Nematode infection in harbour seal (*Phoca vitulina*) at two sites; Sandøy and Hvaler archipelago, Norway

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Master thesis in Marine biology
Department of Biology
Faculty of Mathematics and Natural Sciences
University of Oslo

1st of June 2012

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Summary

The aim of this study was to investigate the species composition of stomach nematodes in harbour seals, and determine if heart- and lung worms are present in the populations in the two areas Sandøy and Hvaler. The results from the study shows great variation in the composition and distribution of the three main species of stomach nematodes in harbour seals, and also revealed that there was nematodes both in the heart and lungs of some of the seals caught. However, no direct conclusions could be made regarding the composition of stomach nematodes; the low number of harbour seals investigated was the reason for this.

Nematodes from harbour seals in Hvaler (Østfold County) and Sandøy (Møre and Romsdal County), were analyzed. The nematodes were collected from the stomach, heart and lungs of the seals. A total of 156 nematodes were found, where four were from the lungs and one from the heart. The species found were *Anisakis simplex, Pseudoterranova decipiens, Contracaecum osculatum, Otostrongylus circumlitus* and *Acanthocheilonema spirocauda*. *P. decipiens* was the species with most representatives in this study with 77 nematodes, followed by 54 *A. simplex* and 20 *C. osculatum*. There were four *O. circumlitus* and one *A. spirocauda.*

There could not be detected any certain trend or pattern for the distribution of the nematode. Due to small sample size the dataset could not be distributed normally. Likewise, statistical significance in difference between the two areas or between the species could not be proven. Hvaler was the area with the highest amount of stomach nematodes with a mean of 38 nematodes. There were only two seals caught in that area. Fives seals were caught in Sandøy with a mean of 15 nematodes. Both the heartworm and the four lungworms were found in seals caught in Sandøy. Considering the economical and ecological importance nematodes in seals have, more emphasis should be put on the nematode faunal distribution and patterns in coastal management. More samples from seals along the coast of Norway could reveal a pattern and explain why the nematode abundance and diversity trends are so variable in harbour seals.

Preface

This master thesis was carried out in 2010 - 2012 at the Department of Biology at the University of Oslo. My principal supervisor was Karl Inne Ugland and co-supervisors Morten Bronndal and Morten Laane.

First of all, I would like to thank my supervisor, Karl Inne Ugland (UiO), for giving me the opportunity to do this master thesis. You have been supportive and optimistic through the entire process. Thank you for all the guidance and quick answers to my e-mails.

Morten (Bronndal), thank you for the wonderful trips to Hvaler and Sandøy. It has been an exciting and educational experience from beginning to the end. You and Gunnar (Gundersen) are responsible for the basis of my thesis, without your good aim and routines I could not have done this thesis. Thanks to Bjørn Berland, who taught me how to identify the nematodes, and for the nice conversations during my visits in Bergen. Thanks to Morten Laane who was prepared to help me in the lab, even though the thesis took a different turn than planned. Thanks to the research group for helping me collect the samples for my thesis; Åsmund Lande, Emma Lähdekorpi and Karin Raamat.

I want to thank my fellow students for making these years valuable and unforgettable; for the many fun and educational fieldtrips, lectures and group seminars. Thanks for the many coffee breaks and good conversations. A special thanks goes to ”Biojentene”, you are amazing.

Kyler, Leigh, Charlotte, Lisa, Norith and Katrine: thank you for taking the time to proofread and comment on my thesis. I want to thank Norith and Lisa for helping me with the statistics as well.

I am grateful for my family and friends for being supportive and patient with me and for always being there for me. Last but not least I want to thank the person who has been there for me everyday, Henrik. You have read, commented and given me ideas for this thesis and believed in me when I didn´t, and for that I am grateful.

Julie Døvle Johansen

Oslo, 01.06.2012

Table of Contents

1 Introduction

1.1 Background

This thesis is part of a larger research program on parasitic nematodes conducted by Karl Inne Ugland at the Marine Biology Research Program of the Department of Biology at the University of Oslo. Samples from harbour seals, (*Phoca vitulina* Linne 1758), and samples from various fish species have been collected by several master students over the past 28 years. Morten Bronndal, head of the Zoological Department at the Department of Biology at the University of Oslo, has been in charge of this part of the study. This particular study was conducted during spring, summer and autumn 2010 and 2011 at the outer Hvaler archipelago in Østfold in southern Norway, and Sandøy archipelago in Møre and Romsdal on the west coast of Norway. Together with Karin Raamat, Emma Lähdekorpi and Åsmund Lande various tissue samples from fish and seals were collected, then nematode parasites were identified in the flesh of the fish, and in the stomach, heart and lung in harbour seals. The nematodes were preserved on 70% ethanol, and the otoliths from the fish were used for age determination. Bjørn Berland, Professor Emeritus, from the University of Bergen advised on the techniques for fixation and identification of the nematodes. The project is ongoing and further results will be presented in 2012.

Of the six species of true seal in the Northeast Atlantic, only harbour and grey seal (*Halichoerus grypus* Fabricius 1791) have permanent residency along the Norwegian coast. Harbour seals are located in sheltered coastal areas along the whole coast of Norway with highest densities in the Møre and Romsdal and Sør-Trøndelag counties. There are three different habitats utilized by harbour seals in Norway: skerries and shallow rocks near the coast, deep fjords and estuarine sandbanks (Bjørge 1991). In Norway, about 6700 harbour seals were recorded in a major survey during 2003 – 2006 (Nilssen and Bjørge 2009). In 1984, the local population around Torbjørnskjær counted approximately 350 animals (Ugland *et al*. 1984). A virus epidemic caused by Phocine Distemper Virus (PDV), in the summer of 1988 reduced the population with approximately 75% (Markussen 1992), but in 2000 the number of animals had increased to about 300 animals (Henriksen and Røv 2004). In 2002, the virus epidemic rallied again (Harding *et al*. 2002), and the population was estimated to be reduced by 75% once again (Morten Bronndal pers. comm. In Jensen 2009). Nilssen and

Bjørge (2009) recorded in their survey 2003-2006 approximately 200-300 harbour seals in the Torbjørnskjær archipelago.

The parasites in seal stomachs are almost exclusively *Anisakis simplex*, *Pseudoterranova decipiens* and *Contracaecum osculatum*. They all belong to the family Anisakidae and have marine mammals as their main host (Anderson 2006). Seal and whale stomachs may contain large amounts of roundworms, and their larvae are found in many types of fish along the coast of Norway (Berland 2003). Their life cycle consists of five different stages and involves two or three middle hosts. Benthic fishes and benthic invertebrates are the intermediate hosts for *P. decipiens*, and sexual maturation and reproduction takes place in seal stomachs (McClelland 1990). In the outer Oslofjord, the harbour seal is the primary final host for sealworms, as grey seals only rarely occur in these areas. At Torbjørnskjær, the seal skerries function as a core area for the distribution of sealworm larvae in fish. Jensen and Idås (1992) found that the sealworm burden in cod decreased significantly with increasing distance from the haul-out-skerries at Torbjørnskjær.

Pseudoterranova decipiens (Krabbe 1878) is known to infect more than 75 fish species in the North Atlantic waters (McClelland 1990; Jensen *et al*. 1994; Desportes and McClelland 2001). As a result of *P. decipiens′* third stage being coiled up in the flesh of codfish and flatfish, a serious extra cost is required from the fishing industry in Norway for removing the nematodes from the fish flesh before the fish can be sold commercially. Along the Norwegian coast the highest nematode prevalence and abundance in demersal fish are due to infection by *P. decipiens.* These include cod (*Gadus morhua*), cusk (*Bromse bromse*), sculpins (*Myoxocephalus scorpius*, Linnaeus 1758), smelt (*Osmerud eperlanus*) and long rough dab (*Hippoglossoides platessoides*) (McClelland 1990; Jensen and Andersen 1992; Jensen 1997). The sealworm has infected such a large fraction of the Norwegian coastal cod, that a specific term for sealworm, Codworm, is commonly used.

Nematodes also infect the heart and lungs in seals. The heartworm *Acanthocheilonema spirocauda* is found in several species of true seals, amongst them the harbour seal (MacDonald and Gilchrist 1969; Dunn and Wolke 1976; Measures *et al.* 1997). The lungworm *Otostrongylus circumlitus* is a large lungworm that occurs in the main bronchi in true seals, amongst them, the harbour seal and grey seal (Anderson 2006). Parafilaroides (*Filaroides*) is another family of lungworms in the suprafamily Metastrongyloidea. The family has four species that is difficult to differentiate by morphology (Gosselin and

Measures 1997). Heavy infections of lungworms and heartworms are considered to adversely affect health and dive skills of the seals, likely having a negative influence on foraging, growth and survival of the seals (Onderka 1989; Bergeron *et al*. 1997b; Gosselin *et al*. 1998; Measures 2003).

Aspholm (1991) worked with nematodes in the outer Oslofjord area as part of a larger project together with Idås (1987) and Jensen (1987) for their Cand. Scient. theses. They documented the degree of infestation in the most preferred prey fishes of harbour seals in the outer Oslofjord. About 70% of cod near the seal skerries were infected with *Pseudoterranova decipiens* and the infection decreased with the distance from the seal skerries. In contrast, *P. decipiens* were only found in 2% of cod sampled 500 meters from the seal skerries. Aspholm (1991) also investigated the nematode infestation in the stomachs of harbour seals from the Oslofjord and Froan area (Sør-Trøndelag county) and grey seals from Froan. Jensen (2009) investigated liver spots and their impact on the general condition of the seals. She also studied the species composition and growth of nematodes in the stomachs. She documented migration of lungworms between different inner organs, but found no health effect caused by the liver lesions.

It is important to build on the knowledge of Idås (1987), Jensen (1987), Aspholm (1991), and Jensen (2009), in order to learn more about nematode composition in harbour seals. The three mentioned researchers identified the nematode composition in fish and seals from the outer Oslofjord, Froan area and Sandøy archipelago. Jensen (1987) and Idås (1987) found that there is little to no nematode presence in fish from areas with low or no occurrence of harbour seals. There is also evidence of decreasing population levels of *A. simplex* since the 1980´s (Jensen 1987; Hansen and Malmstrøm 2006; Jensen 2009). Investigations done by Jensen (2009) showed that *Parafilaroides* sp. and *A. spirocauda* is commonly found in young seals. Studies performed in other areas show that there are large regional differences in the prevalence and abundance of *C. osculatum* and *P. decipiens* (Brattey and Stenson 1993; Stobo *et al*. 2002). Aspholm (1991) found that (1) the total nematode content in harbour seals from Outer Oslofjord in 1984 was greater than documented earlier in Norwegian waters, and (2) that the nematode species composition in harbour seals from the Hvaler area were dominated by *A. simplex*. *P. decipiens* and *C. osculatum* made up less than 10% of the nematodes. The main host for *A. simplex* is whale (Young 1972; Anderson 2006; Berland 2006). Aspholm (1991) and Jensen (2009) found sexually mature *A. simplex* in harbour seals from the Hvaler archipelago. Hansen and Malmstrøm (2006) did not find any sexually mature *A. simplex* in their samples.

Based on the findings of the aforementioned researchers, the targeted area of study involves nematodes from the stomach, heart and lungs in harbour seals from the outer Oslofjord and Sandøy archipelago. This enables comparison of results from the previously mentioned theses, and to determine if there has been a change in trends regarding; abundance, prevalence, sex and stage in *Pseudoterranova decipiens, Anisakis simplex* and *Contracaecum osculatum.* It is desired to investigate if there were sexually mature *A. simplex* in the Hvaler and Sandøy archipelago. In addition to this it is also anticipated to investigate two issues regarding heart- and lungworms; firstly to find out, in my two study areas, whether harbour seals are still infected with heart- and lungworms and secondly to find out whether younger seals have a larger burden of heart- and lungworms. Considering this, focus was on the following issues:

1. Are there any differences in the prevalence, abundance and intensity of *Pseudoterranova decipiens, Anisakis simplex* and *Contracaecum osculatum* in stomachs of seals from Sandøy and Hvaler archipelago?

2. Are there any differences regarding sex, stage and length in *Pseudoterranova decipiens, Anisakis simplex* and *Contracaecum osculatum* in stomachs of seals from Sandøy and Hvaler archipelago?

3. Are there any sexually mature *A. Simplex* in the stomachs of the harbour seals?

4. Are the harbour seals in Sandøy and Hvaler archipelago still infected with heart- and lungworms?

5. Do younger seals have a larger burden of heart- and lungworms?

1.2 Nematode biology

1.2.1 Stomach nematodes

Along the Norwegian Coast, large amounts of these roundworms are found in seal and whale stomachs, their larvae are also found in many fish species (Berland 2003). The nematode species life cycle consist of five different stages and comprise of two or three intermediate hosts. Sexual maturity and reproduction take place in a marine mammal stomach where the two latter stages develop. The eggs and/or larvae are consumed by small crustacean and thereby enter the food web. In fish, the larvae bores their way through the stomach and seeks the inner organs, muscles and liver. The fish reacts in most cases by producing tissue that capsulate the larvae (Berland 1989). In this way the larvae stays there until the next host digests it.

If the host is a fish, the process repeats itself, and it continues until the larvae reach a seal- and whale stomach (Berland 2003). Larger fish species like cod (*Gadhus morhua*) and pollock (*Pollachius virens*) can therefore have enormous amounts of encapsulated nematode larvae, which is often referred to as the seal worm. In marine mammal stomachs the last three nematode stages occur. When seal or whale digest infected fish, the larvae are set free. It grows and changes coat twice before it reaches the sexually mature fifth stage (Anderson 2006). In marine mammal stomach the nematodes can live freely in the stomach, or larvae can bore itself into the stomach wall and create craters with a few or tens of individuals in groups (Aspholm 1991). The female nematode spawns in the marine mammal stomach and the eggs are freed in the sea together with the host’s feces.

Sexually mature *A. simplex* occur mainly in whale (Anderson 2006). Adult individuals are sporadically observed in several seal species, mainly the ringed seal (*Pusa hispida*) and the grey seal (*Halichoerus grypus*), although not in the harbour seal (*Phoca vitulina*) (Davey 1971; Brattey and Stenson 1993; Ólafsdóttir and Hauksson 1998). *A. simplex* follows a pelagic food web (McClelland 2002), and is therefore called herring nematode. Krill (*Euphausiidae*) is often the first host, and larvae are transmitted to different pelagic fish species, or the krill is eaten directly by marine mammals (Figure 1.1) (Anderson 2006). Genetic investigation has in more recent years shown that *A. simplex* consists of five sister species that is so much alike they cannot be separated by morphology (Berland 2003).

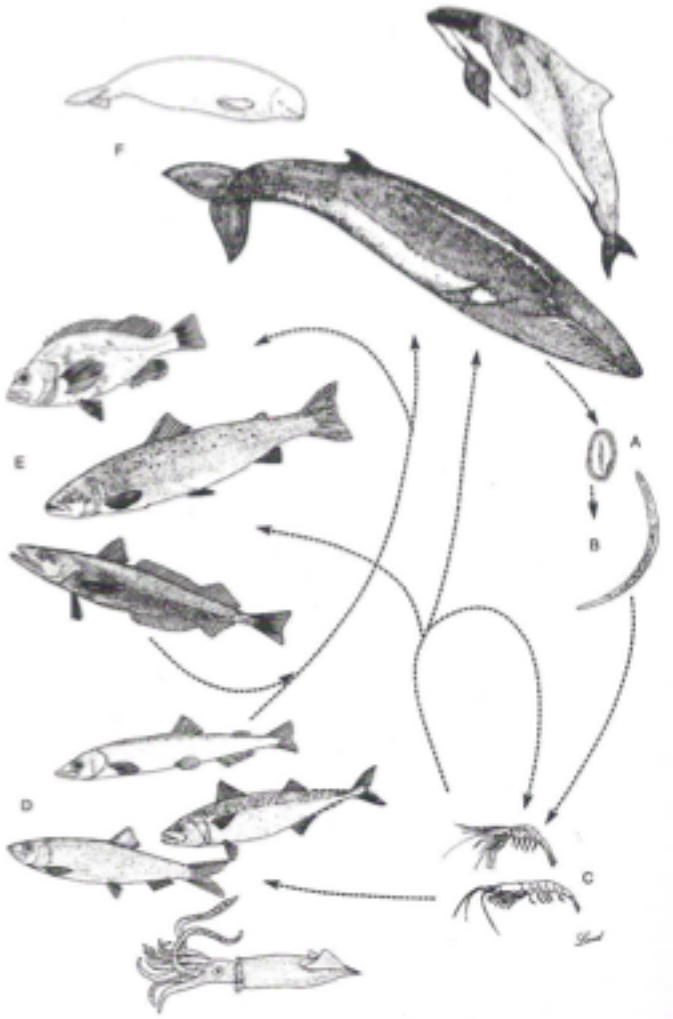


Figure 1.1 Life cycle of *A. simplex*. A=Egg, B=Free-living larvae, C= Crustacean (intermediate host), D and F= Fish and squid (intermediate host), F=Whale (Final host, the nematode matures sexually). (Anderson 2006).

P. decipiens has several seal species as their main hosts, and is the nematode with the highest abundance in coastal seal (Bjørge 1987b; Ólafsdóttir and Hauksson 1998; Stobo *et al.* 2002). Unlike *A.simplex*, the eggs and larvae of *P. decipiens* follow a benthic food web (McCelland 1990; Andersen *et al*. 1995), and is called cod worm. Eggs, excreted with the feces of the seal host, sink to the bottom where they can be consumed by small benthic crustaceans, these are later eaten by fish (Figure 1.2) (McClelland 1990). The larvae can also hatch at the bottom and be eaten directly by fish that are in the seals diet (Myers 1960).

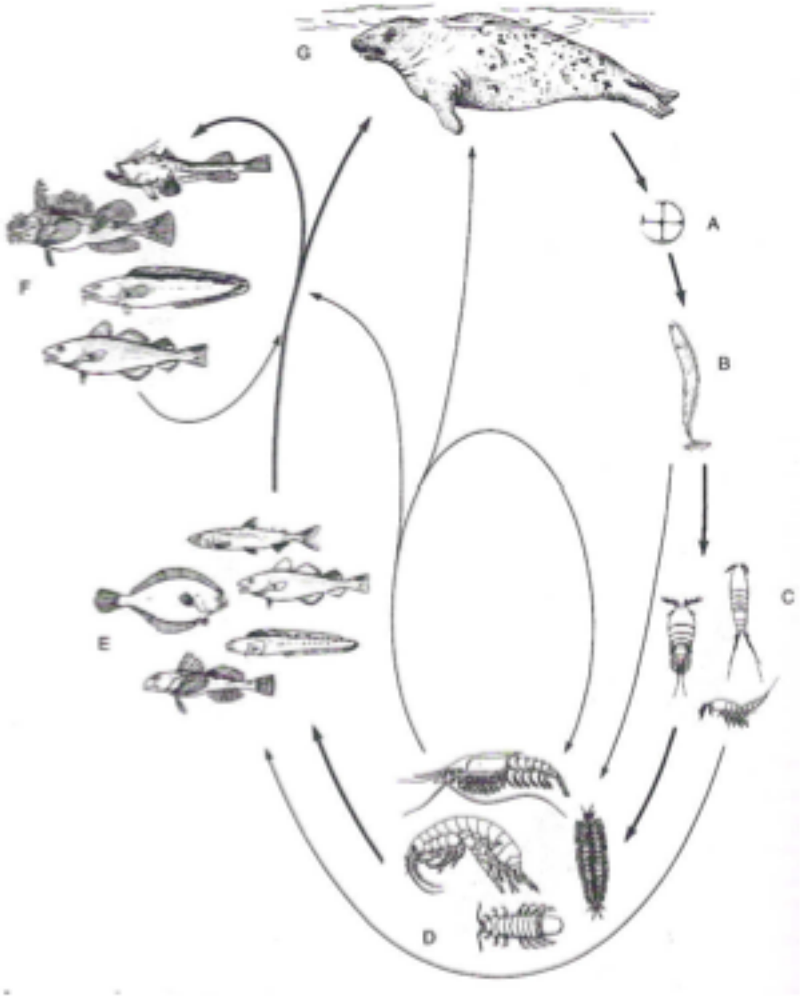


Figure 1.2 Life cycle of *P. decipiens* and *C. osculatum*. A= Egg, B= free-living larvae, C= Small crustacean (intermediate-host), D=larger crustaceans (intermediate-host), E= Primary fishhost, F= Secondary fish host, G= Seal-host (final host, the nematode matures sexually). (McClelland 1990).

Most members of the *Contracaecum* species use piscivore birds as either intermediate host or final host. The exception is *C. osculatum*, which has a life cycle that is similar to *P. decipiens* (Køie and Fagerholm 1995; Anderson 2006). Sexual mature stages of *C. osculatum* occur in several seal species, but the species is far less common than *P. decipiens* in the North-Atlantic (Bjørge 1987; Ólafsdóttir and Hauksson, 1998; Stobo *et al*., 2002). In the northern hemisphere, *P. decipiens* and *C. osculatum* is made up of three and five sister species that has similar morphology and is separated by DNA analysis (Paggi *et al*. 1991; Anderson 2006).

1.2.2 Heartworm

The heartworm, *Acanthocheilonema spirocauda,* is found in several species of true seal, most commonly in the harbour seal (MacDonald and Gilchrist 1969; Dunn and Wolke 1976; Measures *et al.* 1997). Adult specimens from different seal species are roughly the same size (Measures et al. 1997), it is therefore assumed that the heartworm does not have any preferable hosts among the seals, but has equal growth conditions in all species it infects. *A. spirocauda* reproduce with the help of microfilaria (Dailey 1986), and have a vector-born life cycle. Microfilaria's is young larvae in resting stages that is freed in the blood of the seal host, and from there is transmitted between seals through bloodsucking arthropods.

Heartworm in seal is usually situated in the right heart chamber, but is also found in lung arteries and deep in the lung tissue (Measures *et al*. 1997). The harbour seal is most commonly infected with heartworm larvae through the ectoparasite seal lice (*Echinophirius horridus*), which nurture itself on the seal's blood (Leidenberger *et al*. 2007). The seal lice get microfilaria through blood transition of infected seals. It is assumed that the three first larvae stages to *A. spirocauda* develop in seal lice, and that the third stage larvae is transmitted back to the seals blood by seal lice attacks (Geraci *et al*. 1981). The fourth stage of the larvae develops in the blood and matures sexually in the heart of the seal (Figure 1.3).

Heartworm is not found in the grey seal, despite thorough investigations (Measures *et al*. 1997). It is therefore assumed that the grey seal, which in a smaller degree is infected by seal lice than the harbour seal (Leidenberger *et al*. 2007), is not the final host for this parasite. There is strong evidence that a seal attacked by heartworm never gets rid of the infection, and dies as a consequence (Leidenberger *et al*. 2007). This will explain why *A. spirocauda* is mainly found in younger animals, rarely in older, during sampling.

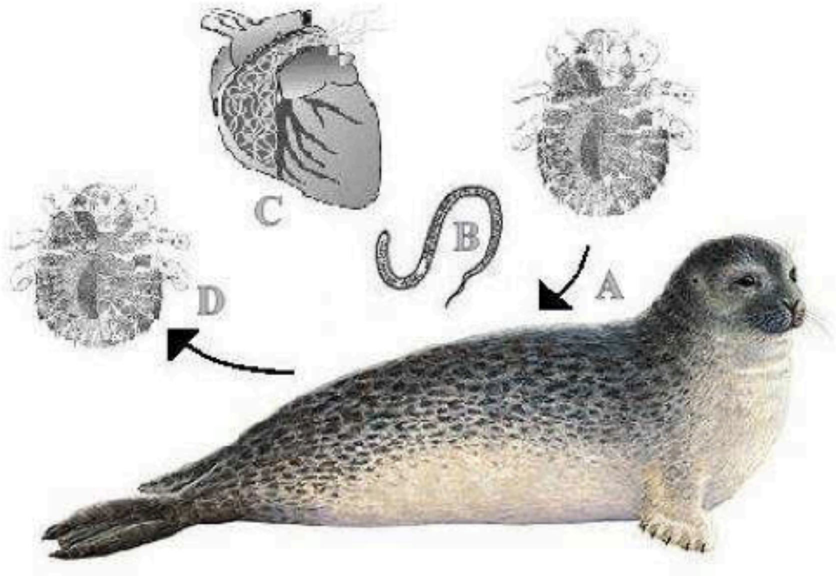


Figure 1.3 Assumed life cycle *A. spirocauda*. Seal is infected with the third stage larvae by bloodsucking seal-lice (A). The larvae develop to the fourth stage heartworm in the blood of the seals (B), before they sexually mature in the heart (C). The microfilaria is freed with the bloodstream. Seal-lice that sucks blood from infected seal, is infected with microfilaria (D), and the three first stages of larvae develops in the seal-lice (Jensen 2009).

1.2.3 Lungworm

The lungworm *Otostrongylus circumlitus* is a large lungworm that exists in the mainstem bronhi and in the bronchi of the true seals, including harbour seals and grey seal (Anderson 2006). *O. circumlitus* is the only species in the family *Otostrongylus* in the suprafamily Metastrongyloidea. The species is ovoviviparous; the eggs develop in the first larval stage in the uterus, and the female lays the larvae directly in the lungs on the seal host. The first larval stage is coughed up by the seal, then swallowed and spread with the feces (Dailey 1986). Further life cycle is not fully known. Studies have shown that fish are necessary intermediate host for *O. circumlitus* larvae, but it is unknown how invertebrates are involved in the species life cycle (Bergeron *et al*. 1997a). It is mainly seals under the age of one year that are infected (Onderka 1989; Bergeron *et al.* 1997b; Gosselin *et al.* 1998). *O. circumlitus* has a circumpolar extent (Bergeron *et al*. 1997a).

Parafilaroides (Filaroides) is another family of lungworms in the suprafamily Metastrongyloidea. The family has four species that are difficult to distinguish by means of morphology (Gosselin and Measures 1997). Compared to *O. circumlitus* the filaroide lungworm is small and is coiled in the lung parenchyma (Dailey 2002). *Parafilaroides* spp. can attach everywhere in the lung tissue, but in adults often attach to the alveoli and in the small bronchi (Onderka 1989; Measures 2001). Females are ovoviviparous and free the first stage larvae in the air tracts. The larvae proceed to passively move up the air tracts with saliva before it is swallowed and is released with the feces of the seal (Measures 2001). The life cycle thereafter is not fully known. However, experiments have shown that fish, and to a lesser degree, also invertebrates, are successful intermediate hosts for the parasites (Measures 2001). It is mainly the species *P. gymnurus* and *P. hispidus* that infect the true seals (Kennedy 1986; Schumacher *et al.* 1990; Borgsteede *et al.* 1991; Claussen *et al.* 1991; Gosselin and Measures 1997; Gosselin *et al.* 1998), *P. gymnerus* is the only species that is known to infect harbour seals. Due to the nematode size and placement in the lung parenchyma, the parasite is difficult to detect, and microscopy is necessary to confirm an infection.

Strong infections of the lung worms *O. circumlitus* and *Parafilaroides spp*. and the heart worm *A. spirocauda* is assumed to infect the health and dive skills of the seals. These nematodes are assumed to have a negative influence on food search, growth and survival of infected animals (Onderka 1989; Bergeron *et al.* 1997b; Gosselin *et al.* 1998; Measures 2003).

1.3 Seal biology

1.3.1 Harbour seal

The harbour seal belongs to the family of true seals (*Phocidae*) which totally consist of 19 species in total. In the North-East Atlantic ocean there are six species of true seals, whereas the harbour seal and the grey seal are the only ones residing along the Norwegian coast. The harbour seal is located near the coast along the entire Norwegian coast, preferring areas surrounded by shallow water. In Norway, there are three different types of habitat which function as resting and pupping sites: skerries near the coast and shallow islets, deep fjords and estuarine sandbanks (Bjørge 1991). The harbour seal usually resides near its resting grounds year-round (Bjørge 1987a). South and South-East of the Norwegian coast, the seal-skerries usually stick above the water through the tidal cycle. On the West coast and in the North of Norway, many seal-skerries are only exposed at low-tide.

Harbour seals reach maturity when they are five or six years old (King 1983), and female mature earlier than males (Bjørge 1992). Females normally have one pup a year and the pupping happens between early June until early July, mainly the time period around 24^{th} of June (Bjørge 1987a). The pup is born with adult fur allowing the seal to enter the water with the mother immediately after birth (King 1983). Female suckle their young for a little less than a month; after this the pups can manage on their own. Change of fur-coat and mating takes place at the end of August and September (King 1983), with their gestation period being approximately seven months. Females have delayed implantation and the fertilized egg starts to grow from one and a half to three months after fertilization (Riedmann 1990). Adult harbour seal have an opportunistic diet-strategy and eat a number of fishes, squids and crustaceans (Härkönen 1987; Olsen and Bjørge 1994).

2 Materials and methods

2.1 Area description

2.1.1 Torbjørnskjær, outer Hvaler

The Hvaler islands consist of approximately 350 islands, located south of Fredrikstad on the eastside of the estuary in the Oslofjord (Figure 2.1 and 2.2) (Staveland 1990). The area has dominantly shallow depths. Approximately one quarter of the seabed is shallower than 6 m, and half of the seabed is at depths less than 20 metres. Glomma is Norway’s longest river, has the greatest water mass and flows into the Oslofjord at Hvaler. The island archipelago, around Torbjørnskjær lighthouse in the outer Oslofjord, consists of seven small islands and several smaller reefs. The islands and reefs are used by harbour seals for resting and pupping (Hansen and Malmstrøm 2006). These islands are situated on a plateau, which is less than 20 meters in depth (Aspholm *et al.* 1995). The substrate is hard-bottom with small and larger rolling stones. Near Heia, south in the area, there is also some soft-bottom substrate (Jensen 2009).

The ocean floor surrounding these islands forms a plateau that follows the direction of the island archipelago. The depth is between 25 and 40 metres. The plateau is approximately 2 km wide near Heia and narrows out near Torbjørnskjær to about 1 km wide. The islands surrounding Torbjørnskjær are cut off from the rest of the Hvaler islands by a 15 km-wide gap (Hansen and Malmstrøm 2006). The characteristics of the waters can lead to large breakers (2 m) at certain wind directions (northeast and east-southeast), at relatively low wind speed (Aspholm 1991). In addition, southerly winds can bring large swells from the North Sea and Skagerrak. The Torbjørnskjær islands are considered heavily exposed (Hansen and Malmstrøm 2006). Counting undertaken in 2008 and 2009, by Jensen (2009), suggests that the population size is about 200 harbour seals in the outer Oslofjord. .

The abundance of harbour seals around Torbjørnskjær is at a minimum level in the winter season, and where the animals stay during this season is not known. Some movement of harbour seals between the Hvaler islands and the west coast off Sweden has been registered (Ugland *et al*. 1984; Markussen 1992), but it is uncertain what impact this has. Grey seals only occur sporadically in the Oslofjord. Individuals of this species are supposed to have migrated from colonies in the Baltic Sea, the coast of Trøndelag and Great Britain.

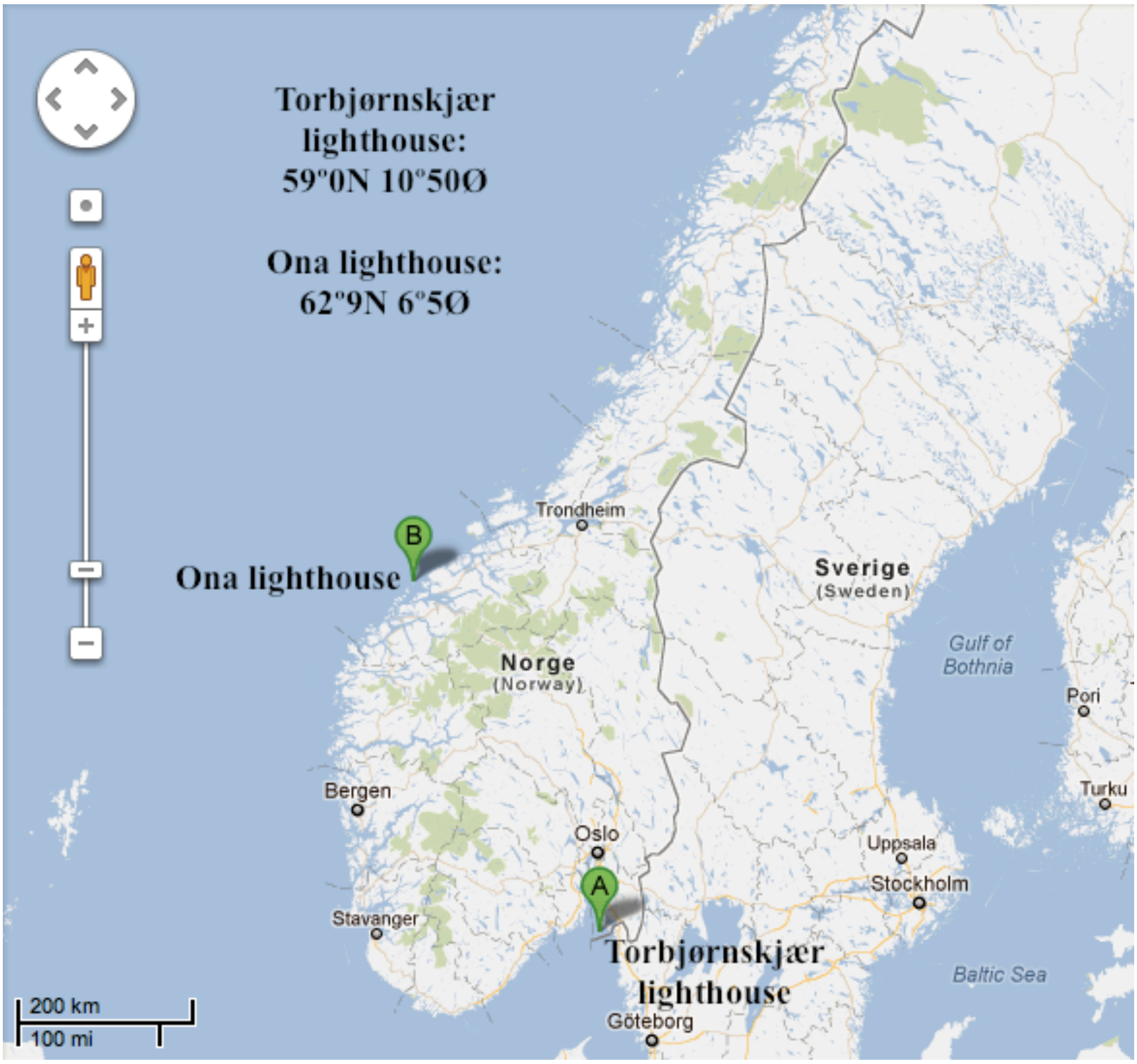


Figure 2.1 Map over Norway, outer Hvaler Islands in the south, Sandøy County in the northwest. The pin marked "**A**" is placed at Torbjørnskjær lighthouse, the pin marked "**B**" is placed at Ona lighthouse (from Google maps with some modifications).

Figure 2.2 The island archipelago around Torbjørnskjær lighthouse (from Google maps with some modifications). The pin marked ”**A**” is Torbjørnskjær lighthouse.

2.1.2 Sandøy

The island archipelago in Sandøy Municipality (Møre and Romsdal County), consists of 800 small islands and reefs (Figure 2.3). Several of the outermost reefs north of Ona lighthouse are important resting and pupping grounds for the harbour seal (Morten Bronnal pers. comm. 2010). Exposed areas have kelp forest (mainly *Laminaria hyperborea*), through which sandy-bottom channels run. Island bays and inlets are predominantly sandy-bottomed. In the deeper areas outside the plateau, the substrate is mostly sand, sludge and mud.

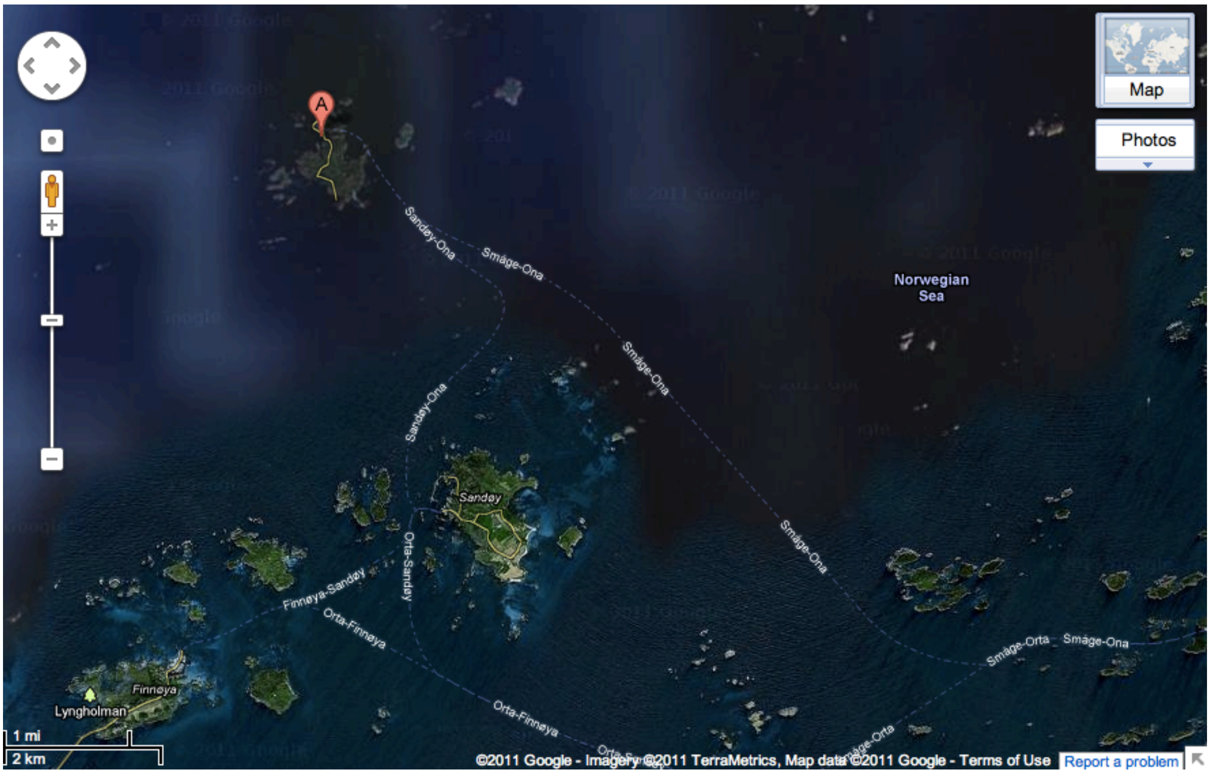


Figure 2.3 Sandøy county island archipelago in Møre- and Romsdal. The pin marked "**A**" is Ona lighthouse (from Google maps with some modifications).

Registrations from the beginning of the 1980´s suggested that the harbour seal population in Møre and Romsdal was 1240. During a state-funded hunting programme in the late 1980´s, 1019 harbour seals where shot and 255 harbour seals were found dead after the virus epidemic in 1988 (Henriksen and Røv 2004). In 1996, after the epidemics, the counting performed using aerial methods suggested the harbour seal population to be 871 (Henriksen and Røv 2004). Conversely the Institute for Marine Science´s (Havforskningsinstituttet) estimate for the time period 2003-2006 there was a mere 200-300 harbour seals in Møre and Romsdal (Nilsen and Bjørge 2009).

2.2 Collection of seal samples

The fieldwork took place June 2010 – September 2011, and the seal hunt took place in two areas. One hunt was at the outermost part of Hvaler, in the area around Torbjørnskjær lighthouse (59°0N 10°50Ø), and the borders for the hunting area were at Torbjørnskjær in the north and Heia nature reserve in the south. The other hunting area was around Ona lighthouse (62°9N 6°5Ø) in Sandøy Municipality.

Two harbour seals were shot close to Torbjørnskjær in outer Hvaler in Østfold. Five harbour seals were shot in Sandøy Municipality in Møre- and Romsdal County. A permit was granted to shoot 20 animals, 10 at each location. Morten Bronndal, head of the Zoological Department, Institute of Biology, at the University in Oslo, was responsible for the hunt. The weapon was a Steyer 30.06 with riflescope and standard hunting ammunition (lead covered point). The boat for transportation to and from the skerries was a 14´Zodiac MK2 with a Johnson 45 hp outboard motor. Sandøy was visited twice; in June 2010 and September 2010. Both expeditions lasted a week. The animals were shot in the sea, then transported to the nearest suitable skerry for dissection and sampling.

Figure 2.4 Dissection of harbour seal (*Phoca vitulina*) in the field. The seal is cut open from the neck to the lower back to measure the blubber thickness. Photo: Julie Døvle Johansen (2010).

The animals were weighed by binding the seal to a rope connected to a scale held up by two people. For seal number two, three, four and five the weight was estimated based upon length and breast circumference. The length of the body was measured from nose tip to tip of tail. The circumference was measured behind the fore-flippers. Chest blubber thickness was measured by placing a measuring band vertically along the blubber in the middle of the fore-flippers (Scheffer 1967). Abdomen blubber thickness was measured similarly on the lower abdomen. The sex was recorded, and the age was estimated from weight and length. The seal fetus from seal number three was frozen and later thawed for measuring and weighing. Inaccurcies are always expected during fieldwork. The age estimation must therefore be seen as an approximate age with 1-2 years margin concerning seals older than five years (Morten Bronndal pers. comm 2012). Seals with a blubber thickness above 25 mm were classified as being in good condition (Drescher 1979; Bäcklin *et al*. 2010).

Sporadical observations and countings done during the fieldwork is described in Appendix B.

2.3 Detection and removing of nematode samples

Liver, lung, heart, stomach and intestinal system were removed, dissected and investigated macroscopically.

2.3.1 Stomach

The stomach was opened either on the skerries or later in the laboratory. When there was little content in the stomach, the content was removed with a pincer and put on Berlands fluid (9.5 parts 100% acetic acid, CH_3COOH and 0.5 parts formaldehyde, CH_2O) to stretch out the nematodes (Berland 1982). After five minutes, the nematodes were transferred over on 70% ethanol where they were stored waiting upon identification. When the stomach was quite full, the content was transferred to Petri dishes to locate all the nematodes. The stomach was put on 70% ethanol, nematodes were later found in the stomach.

Figure 2.5 Stomach from harbour seal (*Phoca vitulina*) with free-swimming nematode. Photo: Julie Døvle Johansen (2010).

2.3.2 Lungs, heart and intestines

The lungs were opened with scalpel on the skerries or in the lab. Scissors were used to cut through to the smallest veins to check for parasites. The same procedure as with stomach nematodes was used when handling the nematodes from lungs, heart and intestine. The same procedure was utilized when handling the hearts and intestines, as they were opened with scalpel and scissors to reach the smallest veins when checking for parasites. Nematodes were collected with pincers. The liver was sampled by making incisions in the surface where lesions were observed, the pieces were collected with a pincer and stored in ethanol.

2.4 Preparation, length measuring and identification of nematodes

Methods for preparation and identification of nematodes were obtained through several meetings and lectures with professor emeritus Bjørn Berland at the University of Bergen.

2.4.1 Preparation

Berland 2005 describes the methods used for preparation of complete nematodes. After identification the nematodes were put in fluids with appropriate refractive index to make them transparent (Berland 1984). Lactophenol (lactic acid, phenol, water and glycerol: 171:1:1:1, refractive index ≈1,44), glycerol – benzyl alcohol (1:1, refractive index ≈ 1,5) and benzyl alcohol (refractive index ≈ 1,54) was applied depending on the size of the individuals (Berland 2005).

The largest individuals were put on acetic acid (CH_3COOH) for 30 minutes before they were transferred to benzyl alcohol (Bjørn Berland pers.comm. 2010). Smaller individuals were put directly in lactophenol or glycerol-benzyl alcohol from 70% ethanol. A Leitz (Wetzlar Biomed) light microscope with magnification 4-40x was used for identification.

The three species of nematode from the family Anisakidae occur in their third, fourth and fifth stages in marine-mammal stomachs. The nematodes were classified down to species, stage and sex for the adult ones. Individuals without lips were regarded as third stage (L3), and an individual with developed lips (labia), but without visible reproduction organs, were regarded as fourth stage (L4). Nematodes were considered to be in the fifth stage if they had clear spicules or eggs present. Males of heartworm *A. spirocauda* have a characteristic spiral at the tip of their tail, and the presence or absence of this, in addition to visible spicules, designates sexually mature males. The presence of egg or larvae designated that they were sexually mature females.

2.4.2 Length measuring

All of the stomach nematodes were measured to the nearest millimetre. The nematodes were carefully stretched out using two pincers and measured on graph paper (Ugland *et al*. 2004). All the measuring was conducted directly after being in fluid (70% ethanol). When comparing length of nematodes it is very important that the nematodes are fixed and treated in the same manner. The nematodes are largely influenced by the chemicals (Fagerholm 1979). After identification, the nematodes were transferred back to 70% ethanol for further storage. The nematode from the heart was measured down to the nearest millimetre, but the four nematodes from the lung were not measured because they were damaged or curled up to heavily to get a precise measurement.

2.4.3 Identification

Anisakis simplex

This species has a large obvious ventricle that is visible like a cavity between the muscular oesophagus and intestine (Figure 2.6). Adult forms of *A. simplex* have a powerful saw-tagged cuticle. The head has three lips and no middle lips. The larvae have a clear bore-tooth that lies in front of the excretion pore, which is in the same height as the start of the oesophagus (Figure 2.7). The larvae-tail is round with a characteristic thin spike at the end (Figure 2.8).

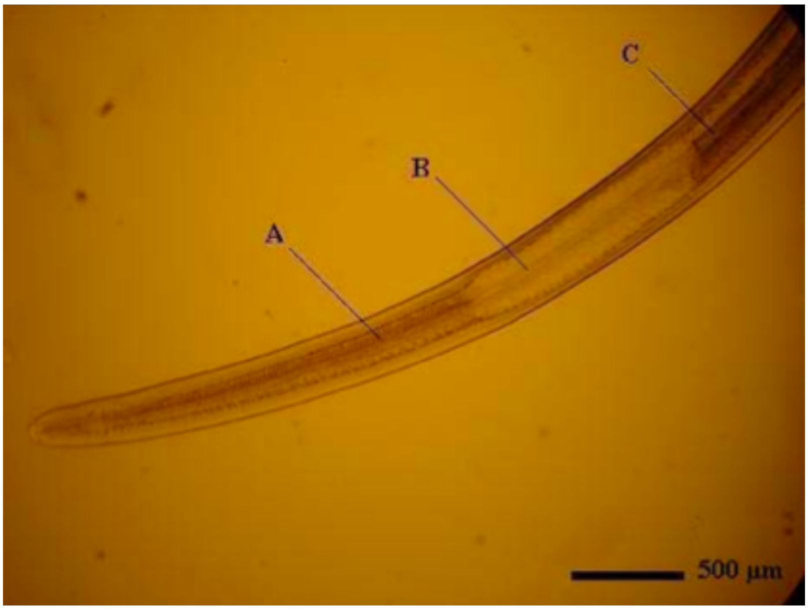


Figure 2.6 Posterior part of *Anisakis simplex*. Notice the clear ventricle (B), between the esophagus (A) and the intestine (C). Photo: Martin Malmstrøm. In Hansen and Malmstrøm (2006).

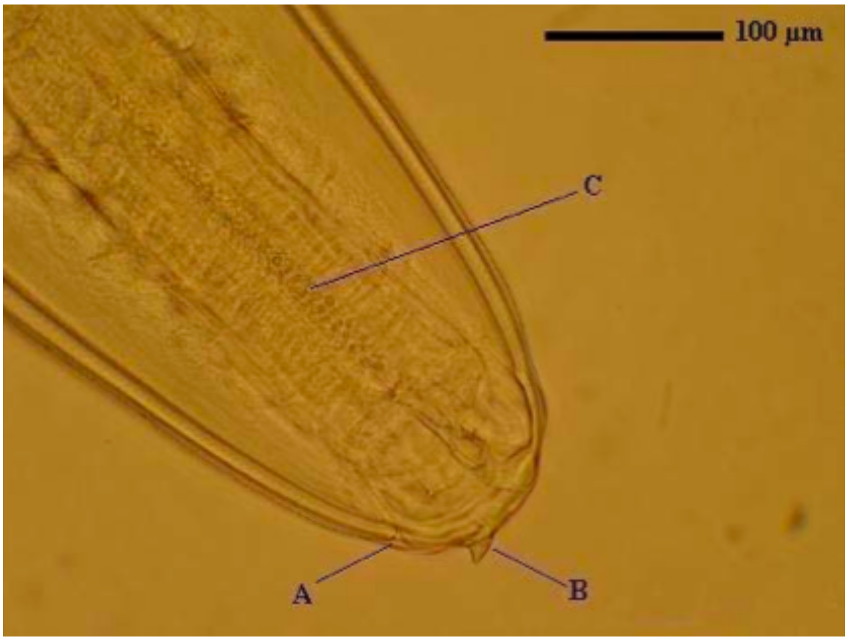


Figure 2.7 The head of *Anisakis simplex*. Notice the large bore tooth (B), the excretion pore (A), and the esophagus (C). Photo: Martin Malmstrøm. In Hansen and Malmstrøm (2006).

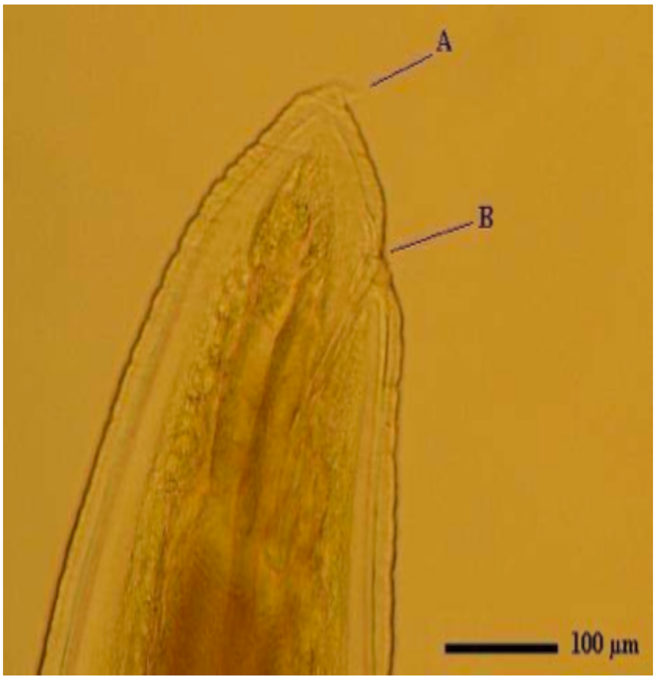


Figure 2.8 The tail of *A. simplex*. It shows the characteristic spike at the tip (A) and the bending between the tip of the tail and the anus (B). Photo: Martin Malmstrøm. In Hansen and Malmstrøm (2006).

Pseudoterranova decipiens

P. decipiens is the sturdiest of the three nematode species. The species has less of a clear ventricle than *A. simplex*. They have intestinal caecum that is visible like a thick sack to the side of the ventricle (Figure 2.9). The species does not have an appendix. The head has three lips, and resembles the head of *A. simplex* (Figure 2.10). The tale is short and heavily curved (Figure 2.11). The bore-tooth in larvae is small, and the excretion-pore is close to the bore-tooth, in the same height as the start of the oesophagus.

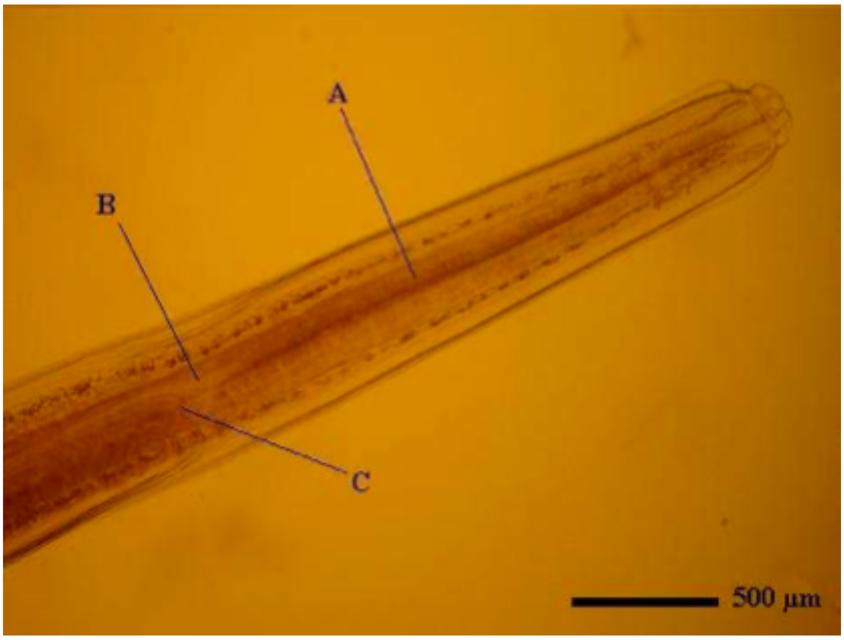


Figure 2.9 Posterior part of *P. decipiens*. Notice the intestinal caecum (C), esophagus (A) and the ventricle (B). Photo: Martin Malmstrøm. In Hansen and Malmstrøm (2006).

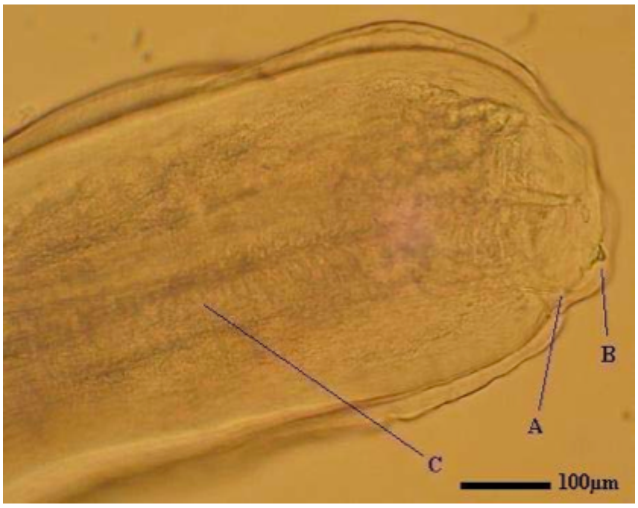


Figure 2.10 The head of *P.decipiens*. It shows the small bore tooth (B), the excretion pore (A), and the esophagus (C). Photo: Martin Malmstrøm. In Hansen and Malmstrøm (2006).

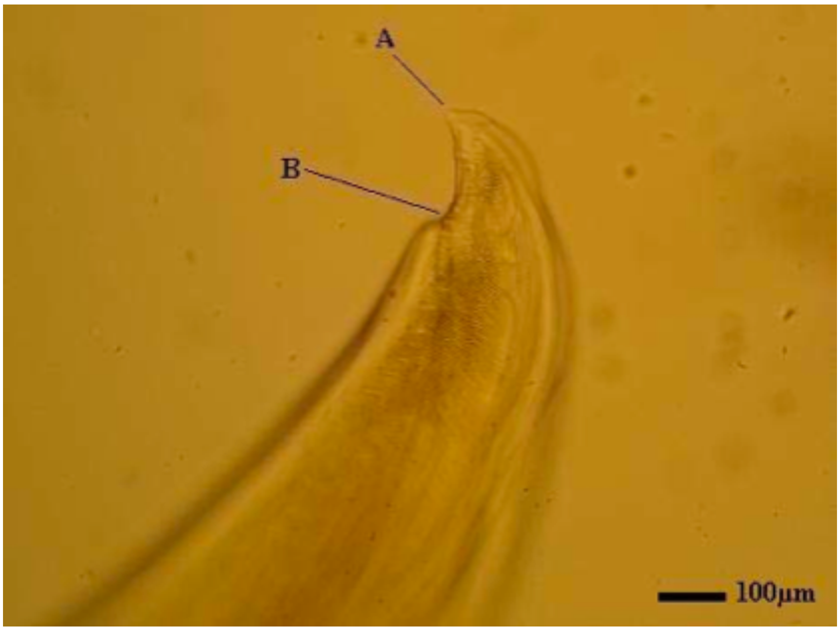


Figure 2.11 The tail of *P. decipiens*. Notice the short, bent tail from anus (B), to the tip of the tail (A). Photo: Martin Malmstrøm. In Hansen and Malmstrøm (2006).

Contracaecum osculatum

The ventricle in *C. osculatum* is considerably smaller than *A. simplex* and *P. decipiens*. The species have both an intestinal caecum and an appendix. The intestinal caecum stretches further towards the head and is somewhat slimmer than the *P. decipiens* intestinal caecum (Figure 2.12). The cuticle close to the head is folded backwards and is visible as a fan-shaped crown around the head. The head holds three lips and three interlabia. The excretion-pore sits directly beneath the bore-tooth on the ventral side, and above the beginning of the esophagus. (Figure 2.13). The tail section is longer than the *P. decipiens* tail, and is heavily curved. The distance from anus to the tip of the tail is long (2.14 mm). The larval bore-tooth is the largest amongst the three species.

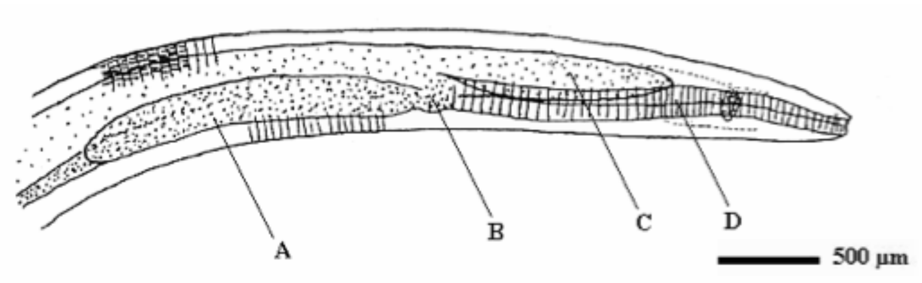


Figure 2.12 Posterior part of *C. osculatum*. Notice the appendix (A), intestinal caecum (C), the small ventricle (B), and the esophagus (D). After: (Berland 2003).

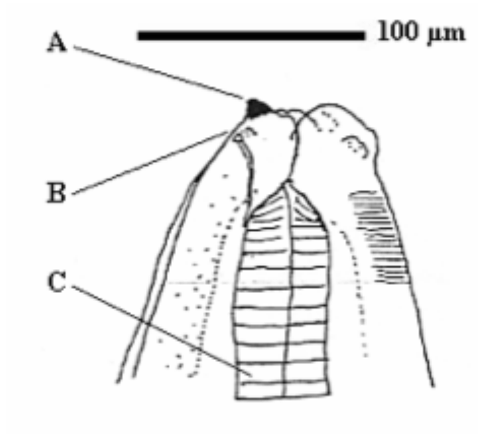


Figure 2.13 The head of *C. osculatum*. Large boretooth (A), excrection pore (B) and oesophagus (C) are marked. After: (Berland 2003).

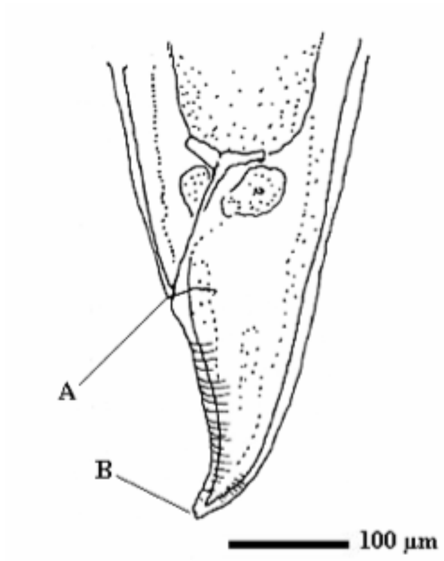


Figure 2.14 The tail of *C. osculatum*. Notice the curving and the long distance from the anus (A) to the tip of the tail (B). After (Berland 2003).

2.5 Statistical methods

All statistical analyses were carried out using Excel for Macintosh 2008. Graphs and tables were made using GraphPad Prism 5 (GraphPad Software inc., San Diego, CA, USA) (Figure 3.2 – 3.7) and Excel for Macintosh 2008 (Figure 3.2 + all Tables). All levels of significance were set to p=0.05 for rejection of H_0:"no difference between Sandøy and Hvaler". Excel for Macintosh 2008 was also used to do the t-tests of the nematode lengths between Sandøy and Hvaler and the Chi-square test for prevalence. The data material in this study was too small to test for normal distribution and equal variances. Therefore a two-tailed t-test with different variances was used. There was also done a chi-square test for the prevalence; this was done in Excel for Macintosh 2008.

3 Results

There is large variability in the data set and many of the frequency distributions are highly skewed. In general samples of less than 50 seals tend to have a non-symmetrical distributions (Karl Inne Ugland pers. comm. 2012).

3.1 Biological information

Seven seals were shot in Hvaler and Sandøy. There was nothing about the seals that would imply poor health condition.

3.1.1 Seal material

Biological data for the seals collected at Hvaler archipelago (two harbour seals) and Sandøy (five harbour seals) is given in Table 3.1.

Table 3.1 Biological data for seven harbour seals. The seal's length is measured from nose tip to tail tip. Circumference is for the chest and is measured directly behind the forearms. Blubber chest is measured by sternum and back in front of the anus. The age for all and weight for four seals is proposed by Morten Bronndal (pers. comm. 2010). As well as the weight of seal number two, three, six and seven.

Seal #	1	2	3	4	5	6	7
Species	P. Vitulina	P. Vitulina	P. Vitulina	P. Vitulina	P. Vitulina	P. Vitulina	P. Vitulina
Area	Sandøy	Sandøy	Sandøy	Sandøy	Sandøy	Hvaler	Hvaler
Date	02.06.2010	03.06.2010	05.06.2010	28.09.2010	29.09.2010	18.08.2010	22.09.2010
Sex	Female	Male	Pregnant female	Male	Male	Male	Male
Estimated age (years)	2	7 - 10 y	10+	3 months	3 months	7 – 8 y	5 – 6 y
Weight (kg)	38,5	80 (est)	112 (est)	21	26	80 (est)	70 (est)
Length (cm)	123	153	170	100	105	145	150
Circumferance cheast (cm)	88,5	101	111	62	72	102	100
Abdominal blubber (mm)	30	32	48	25	29	32	25
Cheast blubber (mm)	28	26	48	25	27	28	30

The catch was two males from Hvaler and two females and three males from Sandøy. Three of the animals were young, probably younger than three years. Two of the seals shot in Sandøy in September, were yearlings, about three months old. One female shot in June in Sandøy was pregnant, likely soon to give birth.

The seal fetus

The seal fetus from seal number three was weighed and measured by Morten Bronndal after it was frozen and thawed. The seal was female and it weighed 6.1 kg and was 76.5 cm from nose tip to tail tip. The circumference was 53 cm. The seal had white, thick fur that 90% of the seal fetus loses before they are born (Morten Bronndal pers. comm. 2012). Harbour seals usually give birth around 24th of June, and the pregnant seal was shot on the 5th of June, implying that the seal fetus was almost fully developed (Morten Bronndal pers. comm. 2012).

3.1.2 The condition of the seals

All of the seals had blubber thickness above 25 mm (except for seal number four with blubber thickness of 25 mm), and was by definition, in good condition (Drescher 1979; Bäcklin *et al*. 2010). Each of their weights were within the normal range observations (Morten Bronndal pers. comm. 2012). The pregnant female had blubber thickness of 48 mm and seemed to be in very good condition.

3.2 Stomach nematodes

The three common stomach nematodes *Anisakis simplex, Contracaecum osculatum* and *Pseudoterranova decipiens* were found in the stomach, rectum and chest cavity of the seals. Not all stages of the three species were represented in the dataset. Stage L5 of *Anisakis simplex* was not represented. Most of the nematodes from *A. simplex* were in L3 and L4. No sexually mature *Anisakis simplex* were found. *Contracaecum osculatum* stage L3 was not represented in the dataset; most of the nematodes of *C. osculatum* were in stage L4 and L5. Most of the nematodes of the species *Pseudoterranova decipiens* were in stage L4 or L5.

When referring to stomach nematodes, nematodes from the chest cavity and rectum are included. It is reasonable to assume that they emerge from the stomach (Karl Inne Ugland pers. comm. 2012).

Table 3.2 Number of nematodes (family Anisakidae) in five harbour seals from Sandøy, in 2010, and two harbour seals from Hvaler, 2010.

Area	Seal #	Amount of nematodes *A. simplex*	%	Amount of nematodes *C. osculatum*	%	Amount of nematodes *P. decipiens*	%	*Total*
Sandøy	1	-	-	-	-	21	100	*21*
	2	7	22.6	-	-	24	77.4	*31*
	3	4	22.2	-	-	14	77.8	*18*
	4	-	-	1	100	-	-	*1*
	5	2	50.0	-	-	2	50.0	*4*
Total	-	*13*	*17.3*	*1*	*1.3*	*61*	*81.3*	*75*
Hvaler	6	18	50.0	11	30.6	7	19.4	*36*
	7	23	57.5	8	20.0	9	22.5	*40*
Total	-	*41*	*53.9*	*19*	*25.0*	*16*	*21.1*	*76*

Seal number one, two, three, four and five came from the Sandøy archipelago. *Anisakis simplex* made up 22.6 % of the nematodes in seal two, 22.2% in seal three, and 50% in seal five. Seal number five had a mere 4 nematodes in the stomach in total. Of the five seals shot at Sandøy archipelago, *A. simplex* made up 17.3% of the total amount of nematodes. *Contracaecum osculatum* made up 100% of the nematodes in seal number six, but no other seals from Sandøy had *C. osculatum* in their stomach. Thus, the *Contracaecum* nematodes from seal six amounted to 1.3% of the total nematode infection at Sandøy. Seal number one had 21 stomach nematodes, all were *Pseudoterranova decipiens*. In seal number two, *P. decipiens* made up 77.4% of the nematodes, in seal number three, 77.8% and in seal number five, 50% of the stomach nematodes. Of the total number of nematodes in collected seals at Sandøy, 81.3% was the Pseudoterranova species (Table 3.2).

Seal number six and seven came from Hvaler archipelago. *Anisakis simplex* comprised of 50% of the stomach nematodes from seal number six. In seal number seven, *A. simplex* constituted 57.5%. *A. simplex* constituted 53.9% of all the stomach nematodes from Hvaler archipelago. *Contracaecum osculatum* comprised of 30.6% in seal number six, and 20% in seal number seven. *C. osculatum* constituted 25% of the stomach nematodes from Hvaler. *Pseudoterranova decipiens* comprised of 19.4% of the nematodes in seal number six, and 22.5% in seal number seven. 21.1% of the total amount of nematodes are from the Hvaler archipelago (Table 3.2).

3.2.1 Prevalence, abundance and intensity

Table 3.3 Prevalence (percentage infected seal), and intensity (mean number of nematodes per infected seal) of *Anisakis simplex* (A. s), *Contracaecum osculatum* (C. o) and *Psedoterranova decipiens* (P. d) in harbour seals from Hvaler and Sandøy archipelago.

Area	Number of seals	Prevalence (%)			Intensity		
		A. s	C. o	P. d	A. s	C. o	P. d
Sandøy	5	60	20	80	4.3	1.0	15.3
Hvaler	2	100	100	100	20.5	9.5	8

The prevalence is the percentage of infected harbour seals. In Sandøy archipelago, *Anisakis simplex* was found in 60% of the seals, *Contracaecum osculatum* in 20%, and *Pseudoterranova decipiens* in 80% of the harbour seals. In Hvaler archipelago the numbers were very different. Only two of the seven seals came from Hvaler, and the prevalence was 100% for the three different nematode species.

By using intensity as a measure, the mean number of nematodes for each infected seal is given, uninfected seals are excluded. In this dataset, the numbers are quite similar as the abundance values. The intensity of *Anisakis simplex* in Sandøy is 4.3, *Contracaecum osculatum* is 1.0 and in *Pseudoterranova decipiens* 15.3. In Hvaler, *A. simplex* mean number per infected seal is 20.5, *C. osculatum* is 9.5 and *P.decipiens* is 8 individuals per infected seal.

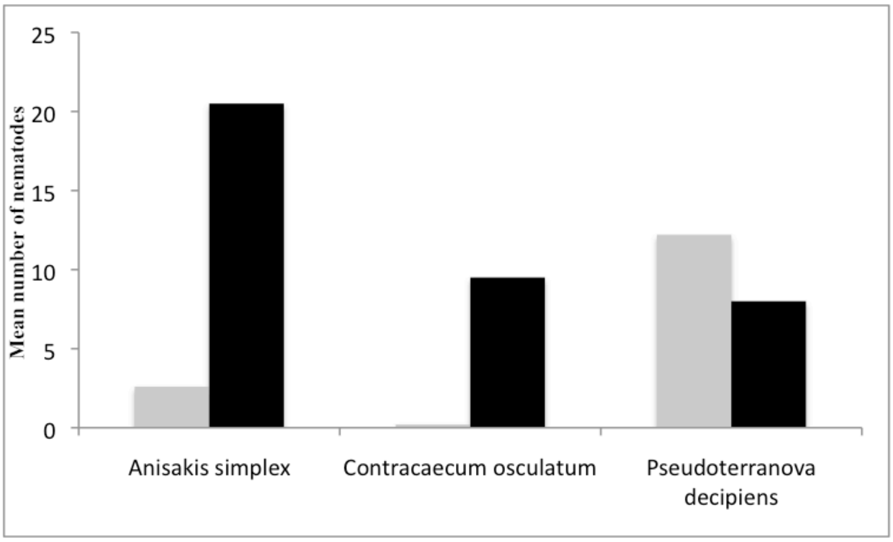


Figure 3.1 abundance (mean number of nematodes for each seal) in Sandøy (grey) and Hvaler (black) archipelago.

The abundance is the mean number of nematodes for each seal. In Sandøy the amount was 2.6 of *Anisakis simplex*, 0.2 individuals of *Contracaecum osculatum*, and 12.2 individuals of *Pseudoterranova decipiens*. In Hvaler, the corresponding mean numbers are of 20.5, 9.5 and 8 respectively.

Chi-square test of prevalence

The prevalences at Sandøy and Hvaler were denoted p_S and p_H respectively. For each nematode species the nullhypothesis was equal prevalence (H_0: $p_S = p_H$). The alternative was different prevalences (H_1: $p_S = p_H$). According to Gotelli and Ellison (2004), we may apply a chi-square test with one degree of freedom:

$c^2 = (a+b+c+d)*(a*d-b*c)^2/(a+c)*(b+d)*(a+b)*(c+d)$

Where a = number of infested seals at Sandøy, b = number of infested seals at Hvaler, c = number of uninfested seals at Sandøy, d = number of uninfested seals at Hvaler.

Since this test statistic has only one degree of freedom the critical value at the 5% level is 3.841, and the significance probability is given as P($c^2 >$ the observed chi square value)

For *Anisakis simplex* (p_S = 60% & p_H = 100%) c^2 = 1.120 and P = 0.290.

For *Pseudoterranova decipiens* (p_S = 80% & p_H = 100%) c^2 = 0.467 and P = 0.495.

For *Contracaecum osculatum* (p_S = 20% & p_H = 100%) c^2 = 3.753 and P = 0.053.

The difference in prevalences were not statistical significant at the 5% level for any of the three species.

3.2.2 Sex, stage and length

Table 3.4 Frequencies of the three stomach nematodes from the harbour seals; *Anisakis simplex* (A.s), *Pseudoterranova decipiens* (P.d) and *Contracaecum osculatum* (C.o), and the stages L3, L4, L5 female and male.

Seal #	A.s L3	A.s L4	A.s L5 Female	A.s L5 Male	P.d L3	P.d L4	P.d L5 Female	P.d L5 Male	C.o L3	C.o L4	C.o L5 Female	C.o L5 Male	Total
1	-	-	-	-	-	2	7	12	-	-	-	-	21
2	-	7	-	-	-	3	12	9	-	-	-	-	31
3	3	1	-	-	-	3	6	5	-	-	-	-	18
4	-	-	-	-	-	-	-	-	-	1	-	-	1
5	2	-	-	-	-	2	-	-	-	-	-	-	4
6	18	-	-	-	-	4	1	2	-	6	3	2	36
7	20	3	-	-	1	2	2	4	-	5	-	3	40
Total	*43*	*11*	-	-	*1*	*16*	*28*	*32*	-	*12*	*3*	*5*	*151*

Infection of nematodes varied between one and 40 in the seals. Seals number one, two, three, six and seven had between 18 and 40 stomach nematodes. Seals four and five had respectively one and four stomach nematodes. They were yearlings (approximately three months) and had less exposure to nematode infections than the five older seals. *A. simplex* L5 and *C. osculatum* L3 were not found in any of the seals. Seals one and three had the smallest amount of nematodes of the adult harbour seals.

The variance in the dataset was substantial making it difficult to analyze the dataset statistically (Karl Inne Ugland pers. comm. 2012).

Pseudoterranova decipiens was by far the most abundant species in my dataset. The third stage occurred only in one individual, the fourth stage had 16 occurrences and the fifth was had 28 females and 32 males. 77 samples of *P. decipiens* were found in total.

Table 3.5 Number of individuals of the three stages (L3, L4 and L5) of *Anisakis simplex, Contracaecum osculatum* and *Pseudoterranova decipiens* in harbour seals from the Hvaler and Sandøy archipelago. A graphical presentation in Appendix C.

Species	*Anisakis simplex*						*Contracaecum osculatum*						*Pseudoterranova decipiens*					
Area	3rd st.	%	4th st.	%	5th st.	%	3rd st.	%	4th st.	%	5th st.	%	3rd st.	%	4th st.	%	5th st.	%
Sandøy (n=5)	**5**	**38,5**	**8**	**61,5**	**0**	**0**	**0**	**0**	**1**	**100**	**0**	**0**	**0**	**0**	**10**	**16,4**	**51**	**83,6**
Hvaler (n=2)	**38**	**92,7**	**3**	**7,3**	**0**	**0**	**0**	**0**	**11**	**57,9**	**8**	**42,1**	**1**	**6,3**	**6**	**37,5**	**9**	**56,3**

Table 3.5 shows the amount of individuals of the three stages of *Anisakis simplex*, *Contracaecum osculatum* and *Pseudoterranova decipiens* in harbour seals from the Hvaler and Sandøy archipelagos. From Sandøy, the different stages of *Anisakis simplex* are distributed like this: third stage (L3) 38.5%, fourth stage (L4) 61.5% and none of stage five (L5). *Contracaecum osculatum*; L3 0%, L4 100%, L5 0%. *Pseudoterranova decipiens*; L3 0%, L4 16.4% and L5 83.6%.

In Hvaler, the different stages of *Anisakis simplex* have the following distributions: L3 92.7%, L4 7.3% and none of L5. *Contracaecum osculatum*; L3 0%, L4 57.9% and 42.1% of L5. Pseudoterranova decipiens; L3 6.3%, L4 37.5% and L5 56.3.

Length measurements of stomach nematodes

The lengths of the stomach nematodes from the harbour seals from Sandøy and Hvaler archipelago were split into the two corresponding groups, and then graphically displayed. The data that make up the basis for these graphs are described in Appendix D.

Sandøy archipelago

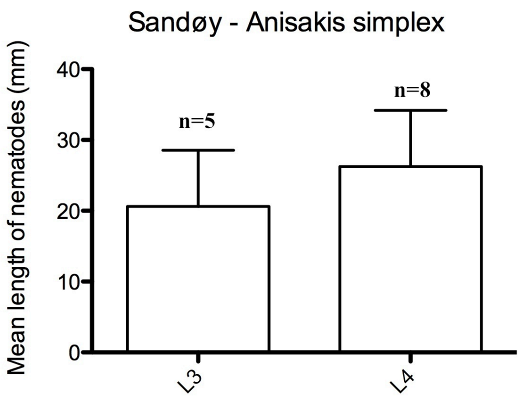


Figure 3.2 Mean lengths (mm) of *Anisakis simplex* stage L3 and L4 in Sandøy including number of nematodes (n) and standard deviation (line above column).

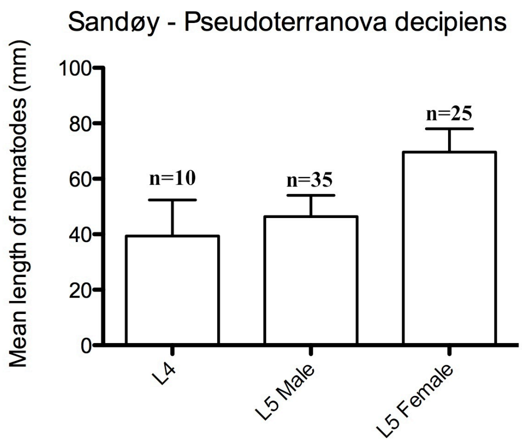
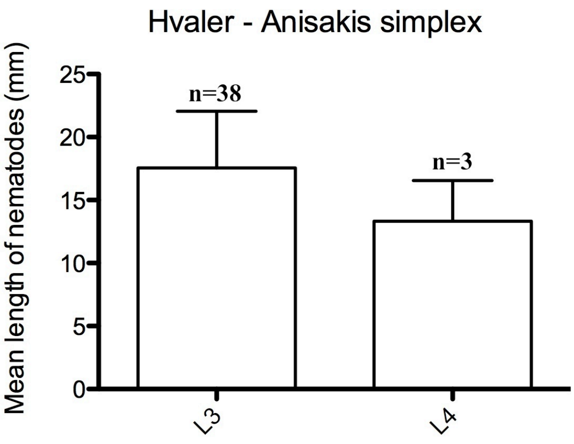


Figure 3.3 Mean lengths (mm) of *Pseudoterranova decipiens* stage L4, L5 male and female in Sandøy including number of nematodes (n) and standard deviation (line above column).

Figure 3.2 and 3.4 show the mean length of nematodes. *Anisakis smplex* has mean body length of 20.6 mm (n=5, SD=8) in stage L3 and a mean body length of 26.3 mm (n=8, SD=7.9) in stage L4. *Pseudoterranova decipiens* L4 has a mean length of 39.4 mm (n=10, SD=13). The mean body length of L5 Male was 46.4 mm (n=35, SD=7.7), and female stage L5, 69.6 mm (n=25, SD=8.4). There was only one *Contracaecum osculatum*, it was in stage L4 and was 11 mm long.

Hvaler archipelago



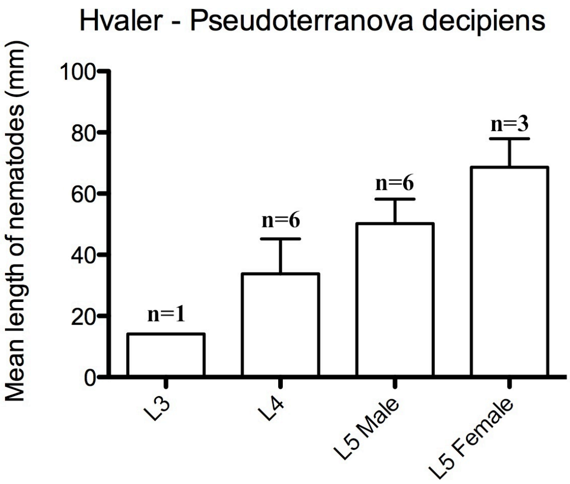


Figure 3.4 Mean lengths (mm) of *Anisakis simplex* in stage L3 and L4 in Hvaler including number of nematodes (n) and standard deviation (line above column).

Figure 3.5 Mean lengths (mm) of *Pseudoterranova decipiens* stage L3, L4, L5 male and female in Hvaler including number of nematodes (n) and standard deviation (line above column).

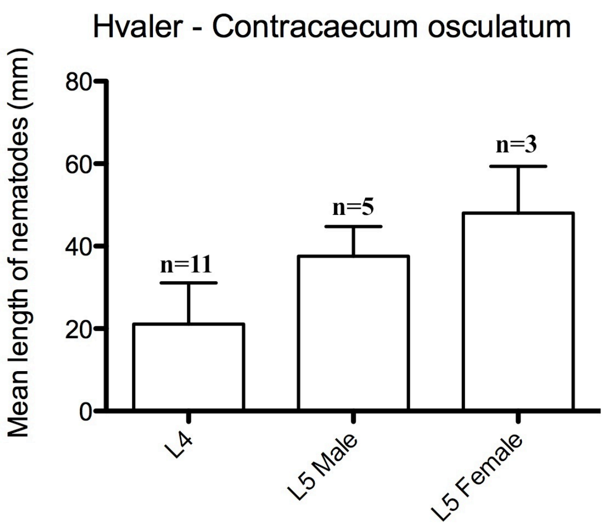


Figure 3.6 Mean lengths (mm) of *Contracaecum osculatum* stage L4, L5 male and female in Hvaler including number of nematodes (n) and standard deviation (line above column).

The nematodes *Anisakis simplex* in stage L3 from stomachs of harbour seals from Hvaler archipelago has a mean body length of 17.6 mm (n=38, SD=4.5). In L4 the mean body length was 13.3 mm (n=3, SD=3.2). *Pseudoterranova decipiens* stage L3 had only one representative and was 14 mm long. Mean length L4 was 31 mm (n=6, SD=12.8), L5 Male 50.2 mm (n=6, SD=8) and L5 female mean length was 68.6 mm (n=3, SD=9.3). The mean body length of stage L4 of *Contracaecum osculatum* was 21.1 mm (n=11, SD=10), L5 male was 37.6 mm (n=5, SD=7.2) and mean body length of L5 female was 48 mm (n=3, SD=11.4).

T-tests of nematode lengths

H_0= Sandøy and Hvaler have nematodes of similar length

H_a= Sandøy and Hvaler have nematodes of differing length

The mean length of *Pseudoterranova decipiens* in stage L4 was 39.4 mm (SD=13) in Sandøy and 31 mm (SD=12.8) in Hvaler, the the t-test indicated that there were no significant difference between Sandøy and Hvaler (P=0.388). *P. decipiens* L5 male mean length in Sandøy was 46.4 mm (SD=7.7) and in Hvaler 50.2 mm (SD=8), again the t-test indicated no significant difference between the two areas (P=0.321). *P. decipiens* L5 female mean length in Sandøy was 69.6 mm (SD=8.4) and in Hvaler 68.6 mm (SD=9.3). There were no statistical significant difference her either (P=0.881).

Anisakis simplex L3 had mean length of 20.6 mm (SD=8) in Sandøy and in Hvaler the mean length was 17.6 mm (SD=4.5). The t-test showed that there was no difference between the two areas (P=0.445). *A. simplex* stage L4 had mean length of 26.3 mm (SD=7.9) in Sandøy and 13.3 mm (SD=3.2) in Hvaler, and was different in the two areas (P=0.004).

The species *Contracaecum osculatum* was not testet with a t-test; it was only represented in stage L5 male and L5 female in Hvaler, stage L4 was only found once in Sandøy. *P. decipiens* stage L3 was not testet either; it was only found in Hvaler.

3.3 Nematodes in the heart and lungs

All the seals were examined for heart- and lungworms. One seal from the Sandøy archipelago had four *Otostrongilus circumlitus* in the lungs. A different seal at the same site had one heartworm, *Acanthocheilonema spirocauda.*

3.3.1 Heartworms

Acanthocheilonema spirocauda was found in seal number five from Sandøy archipelago. This gives a prevalence of 20% in Sandøy, and 14.3% in Sandøy and Hvaler archipelago. The seal was a male yearling (approximately three months), and not sexually mature. The nematode was 91 mm long. Microscope pictures of heartworm are shown in Figure 3.7. The seal was in good health with blubber thickness of 29 mm (abdominal blubber) and 27 mm (chest blubber).

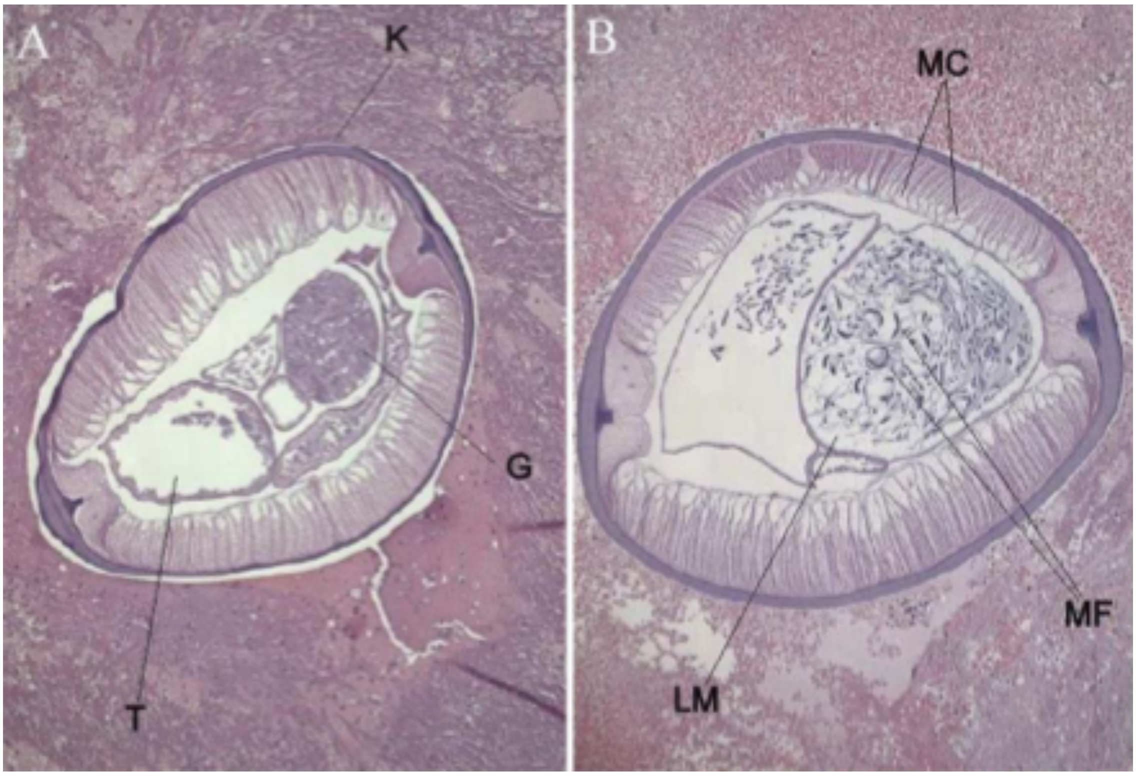


Figure 3.7 The heartworm *Acanthocheilonema spirocauda* in a young male harbour seal. A: Cross-section of adult *A. spirocauda* in coagulated blood in the right ventricle of the heart. Cuticle (K), gonads (G) and intestines (T) are marked. 100x magnification. B: Adult female *A. spirocauda* with microfilaria (MF) in the uterus (LM). Muscle cells (MC) are marked. 100x magnification. Photo: Turid Vikøren Veterinary Institute Oslo (2009), in Jensen (2009).

3.3.2 Lungworms

Two lungworm species occur in seals in the North Atlantic, *Otostrongilus circumlitus* and *Parafilaroides gymnurus* (Measures 2001). Four individuals of the worm *Otostrongilus circumlitus* was in seal number four from Sandøy archipelago. The seal was approximately three months old and male. It showed no apparent signs of poor health and the blubber thickness was good (25 mm abdominal blubber and 25 mm chest blubber) (Drescher 1979; Bäcklin *et al*. 2010). The nematodes were extracted with pincers from the bronchi. The four nematodes were of different size, two small and two large. Three individuals were not complete, but Bjørn Berland managed to look at the essential body parts to determine the species. The charismatic claw-tail was clearly seen (Bjørn Berland pers. comm. 2012). Only one seal had lungworms, the prevalences are the same as for heartworm, since only one seal had heartworm and they were both collected at Sandøy.

For table over the lungworm findings, see Appendix E. For complete dataset, see Appendix A.

4 Discussion

4.1 Biological information

All seven seals had a blubber thickness above 25 mm (Table 3.1). Drescher (1979) and Bäcklin *et al*. (2010) classified a blubber thickness above 25 mm as an indicator of good health. The blubber thickness of seal is normally at its lowest during the time of reproduction and moulting (Drescher 1979; Pitcher 1986; Nilssen *et al*. 1997). The time of reproduction and moulting is roughly between 10th of July and 20th of August (Morten Bronndal pers. comm. 2012). Only one of the seals in this thesis (seal six) was caught within this time period, on August 18th 2010.

During the fieldwork no observations of any sick or malnourished seals were made. No dead or malnourished pups were encountered around the seal skerries during the birthing or weaning periods. Generally the harbour seals at the two sites seemed to be in good condition in the summer months. The weight and measurements also confirm this. The seals in this study were caught close to the season for reproduction and moulting, a period when harbour seals generally have less blubber than the rest of the year (Drescher 1979; Nilssen *et al.* 1997). When this is considered, the condition of the seals in this material was well within the normal range.

4.2 Stomach nematodes

4.2.1 Prevalence, abundance and intensity

Previous investigations of harbour seals from different areas along the Norwegian coast, show that the mean number of nematodes in the stomachs varies between populations annually (Benjaminsen *et al*. 1978; Bjørge 1987b). In British waters Young (1972) found a mean of 80 nematodes in 19 harbour seals. Wootten (1973) found a mean of 189 nematodes in seven harbour seals from the East coast of Scotland. In the area around Nova Scotia, Canada, Scott and Fisher (1958) found a mean of 72 nematodes amongst 276 harbour seals gathered from several areas. One of the areas (Bras d´Or Lakes) had a mean of 182 nematodes. The amounts of nematodes found in this study were quite low compared to the studies mentioned above. The observed variation from population to population (Scott and Fisher 1958; Benjaminsen *et* *al*. 1978), and the annual variation (Bjørge 1987b), can provide reason to believe that large variation in nematode infection in Harbour seals is common. The results in this study also show large variation.

The prevalence (percentage of infected seal) of *Pseudoterranova decipiens, Anisakis simplex* and *Contracaecum osculatum* in seals caught in Hvaler was 100% for all three species (Table 3.3). However, this might be a result of randomness as the sample size was only two seals in Hvaler. In regards to the five seals that were caught in Sandøy, the prevalence was 60% *Anisakis simplex*, 20% *Contracaecum osculatum* and 80% *Pseudoterranova decipiens*. Also in Sandøy the sample size was small (five seals); this result may also be due to a small sample size.

The abundance (mean number of nematodes in all seals) of *A. simplex* in Hvaler archipelago in the study, was 20.5 individuals (Figure 3.3). This was the highest number of all of the nematode species and areas in the study. The second highest amount of nematodes came from *P. decipiens* in Sandøy with 12.2 individuals. *C. osculatum* was represented in Hvaler archipelago with 9.5 individuals, in Sandøy the amount was a mere 0.2 individuals. Aspholm (1991) found that *C. osculatum* had an abundance of 3.2 and *P. decipiens* 9.4 in the 29 seals caught in Hvaler.

Intensity is a measure of the mean number of nematodes for each infected seal. Aspholm (1991) found the intensity of the three nematode species in harbour seals in Hvaler to be 195.1 for *A. simplex*, 4.7 *C. osculatum* and 24.8 for *P. decipiens*. In this study the intensity in Sandøy archipelago was 4.3 (*A. simplex*), 1.0 (*C. osculatum*) and 15.3 (*P. decipiens*). For Hvaler the numbers were 20.5 (*A. simplex*), 9.5 (*C. osculatum*) and 8.0 (*P. decipiens*).

There currently appears to be no existing comparative studies completed on prevalence, abundance and intensity in Sandøy and Hvaler. Investigations from other areas have shown that there are large regional differences in abundance and prevalence regarding *C. osculatum* and *P. decipiens* (Brattey and Stenson 1993; Stobo *et al*. 2002). Brattey and Stenson (1993) found that 70% and 90% of the harbour seals from the Netherlands and Newfoundland were infected with *C. osculatum* and *P. decipiens*. The infection rate of *P. decipiens* was extraordinarily high with a mean of 126.7 nematodes per seal. In Sable Island, Stobo *et al*. (2002), found that *C. osculatum* had both low prevalence (14.5%) and low abundance (0.3) in the seals. This underlines further that there are large variances in abundance and prevalence regarding *C. osculatum* and *P. decipiens*. In this study the numbers are not statistically significant.

Five harbour seals were collected from Sandøy; three of these were infected with *A. simplex*, one was infected with *C. osculatum* and four with *P. decipiens* (Table 3.2). These results are quite different from Aspholm (1991), which found that almost 95% of the nematodes found in harbour seals from Froan (west coast of Norway) in 1985, were *P. decipiens*. Bjørge (1987b) found the following species composition in harbour seals from the west coast of Norway; 26.2% *A. simplex*, 7.8% *C. osculatum* and 66% *P. decipiens*. In this study the distribution from Sandøy was; 17.3% *A. simplex*, 1.3% *C. osculatum* and 81.3% *P. decipiens*. In relation to Bjørges ´(1987b) findings and the results from this study, the results are not too different regarding which species dominate and which species has the lowest percentage in the seals. Aspholm (1991) had four seals and there were five seals in this study, from the west coast of Norway. Neither this study, nor Aspholm´s (1991) material from the west coast of Norway is large enough to compare the species distribution with what has been observed in other investigations.

Considering the nematode infections overall, the five harbour seals from Sandøy archipelago had a mean number of 15 (75/5=15) nematodes per seal (Table 3.2). The two seals from Hvaler archipelago had a mean number of 38 (76/2=38) nematodes per seal (Table 3.2). *P. decipiens* made up 81.3% of all the nematodes from Sandøy archipelago and 21.1% of the nematodes from the Hvaler archipelago. Aspholm (1991) found a much higher infection rate in the seal stomachs; in 1984 he registered a mean of 194 nematodes. He also found that *Anisakis simplex* made up 77% with the highest abundance of 181.7. Twenty-nine seals were investigated by Aspholm (1991) in the Hvaler archipelago, in the outer Oslofjord. In this study there were only two harbour seals from the same area. According to Jensen (1987), 66% of cod in the outer Oslofjord was infected with larvae of *A. Simplex*, with a mean of 2.4 larvae per cod. Hansen and Malmstrøm (2006) found that only 6% of cod were infected with *A. Simplex* in the same area, with a mean of 0.24. The decline in amount of *A. simplex* in cod from the outer Oslofjord from the middle of the eighties to 2006 might explain some of the decline in *A. simplex* in harbour seals from the Hvaler archipelago in the same time period. In Hvaler, *A. simplex* made up 50% of the nematodes in seal six of this study, and 57.5% in seal seven (Table 3.2). The total amount of nematodes in these seals were 36 and 40; in other words, the infection rate was quite low compared to Aspholm (1991) findings.

Aspholm (1991) found that a large fraction of the seals were infected with *C. osculatum* (69%), the abundance of this species was 3.2 per seal. Hansen and Malmstrøm (2006) did not find any *C. osculatum* in their four harbour seals from the Hvaler archipelago examined in 2004/2005. *C. osculatum* was found in seal number four from Sandøy archipelago in this study´s dataset. It was the only nematode discovered in that particular seal (Table 3.4). *C. osculatum* was also found in both seals from the Hvaler archipelago, in seal six (11 individuals) and in seal seven (eight individuals), making up 30.6% and 20% of the nematodes in those seals (Table 3.2).

P. decipiens was found in four of five seals from Sandøy archipelago (prevalence 80%), and in both seals from the Hvaler archipelago (prevalence 100%). The infection rate varied between two and 24 individuals, and the abundance rate was 12.2 in Sandøy and eight in Hvaler. In Aspholm (1991) the prevalence of *P. decipiens* was somewhat lower with 38% in Hvaler. Of the 29 collected seals Aspholm (1991) studied from the Hvaler area, only 11 of these were infected with *P. decipiens*; However he found great variability in the degree of infection. 10 of 11 of infected seals had one to four nematodes, while one seal had 255 individuals of *P. decipiens* in the stomach.

If one overlooks the one outlier seal with large amounts of *P. decipiens* in the stomach, Aspholm dataset from his thesis of 1991, had an abundance of 0.6 nematodes per seal. This shows that the amount of nematodes in the seals had great variability. Hansen and Malmstrøm (2006) found *P. decipiens* in three of the four investigated seals from 2004/2005 with an infection rate between 8 and 44 individuals per seal and an abundance of 15.3. The numbers from this study are more similar to Hansen and Malmstrøms´ (2006) numbers than Aspholm (1991). The one harbour seal from Aspholms´ (1991) study with 255 *P. decipiens*, have not proven to be a typical individual. Perhaps there were something in the seals diet or preferred nutritional areas that contained an abnormally high amount of nematodes. Or it might be that this particular seal had extremely good growth conditions for the stomach nematodes. There is no data material to confirm or reject this, so it is just speculations.

Lick (1989) investigated 274 (self) dead harbour seals from the Wadden Sea outside Schleswig-Holstein and the Danish west coast. Of the 4953 nematodes Lick (1989) investigated, about 95% were *P. decipiens*, about 3% *C. osculatum* and about 1% where *A. simplex*. Lick (1989) did not find any significant differences in the relative abundance of the three nematode species between the two sexes. Lunneryd (1991) found 8.3% *A. simplex*, 20.7% *C. osculatum* and 71% *P. decipiens* in the nematode fauna of 165 (self) dead harbour seals from the west coast of Sweden. The nematode species composition was approximately the same in harbour seals from different areas of the North Sea during the seal plague in 1988 (Lunneryd 1991). There were small amounts of *A. simplex* found in the harbour seals during the seal plague (Aspholm 1991). Aspholm (1991) further suggests that the low percentage of *A. simplex* found during the plague of 1988 can be explained by the harbour seals vomiting when they got sick, or that *A. simplex* has a quicker passing time in the intestines of harbour seals than the two other nematode species, in a combination with low food intake before the seal died. Aspholm (1991) states that this thought is supported by the species composition with *A. simplex*. Aspholm (1991) found a relationship between stage L3, L4 and L5 to be 8:7:1 before the plague of 1988 and 1:2:0 after. Lunneryd (1991) found the relationship to be 1:5:0 in Swedish waters during the seal plague.

Chi-square test of prevalence

The chi-square test showed that there were no statistical significance at the 5% level for any of the three species *A. simplex*, *P. decipiens* and *C. osculatum*. The nullhypothesis (H_0: p_S = $p_{H)}$ was not rejected. These results must be seen in the light of the small sample size. Since this test only had one degree of freedom at the 5% level, the critical observed chi-square value was 3.841. *A. simplex* showed values for X^2 = 1.120 and P=0.290, *P. decipiens* got values of X^2=0.467 and P=0.495, and *C. osculatum* got values of X^2=3.753 and P=0.053. The prevalence of *C. osculatum* was 20% in Sandøy and 100% in Hvaler. The significance probability was 5.3% and thus boarder of being significant.

4.2.2 Variation in nematode sex, stage and length

The average length of a nematode reflects how well the species is adapted to the host, and may be used as a measure for the spawning potential in the host (Ugland *et al*. 2004). Chemicals and the way the nematodes are handled during sampling will influence the length of the parasites in different ways (Fagerholm 1979). Comparing samples and authors should be done with caution.

In Sandøy the mean length of *A. simplex* in stages L3 and L4 were 20.6 mm and 26.3 mm respectively (Figure 3.2). *A. simplex* in Hvaler had mean lengths of 17.6 mm (L3) and 13.3 mm (L4). Aspholm (1991) found mean lengths of third stage *A. simplex* in harbour seals from

Hvaler to be 20 mm. The length of *A. simplex* (L3) in fish is stated to be everything beween 13 and 36 mm (Punt 1941; Oshima 1972; Smith and Wootten 1984). Jensen (1987) found mean lengths of *A. simplex* in cod from Hvaler to be 21 mm. The third stage in cod is not far from the third stage in harbour seals from Sandøy in this study. Aspholm´s (1991) *A. simplex* nematodes from harbour seals and grey seals indicates low growth rate in the fifth stage. This is consistent with the general perception that seals are less suitable than whales as hosts (van Thiel 1966; Young 1972; Smith and Wootten 1978). In the t-test *A. simplex* L3 showed no difference between the two areas (P=0.445). *A. simplex* in stage L4 showed that there was significant difference between the two areas (P=0.004). However, there were only eight individuals from Sandøy and three individuals from Hvaler, so the sample size is too small to draw any conclusions from the t-test.

The mean length of *P. decipiens* in Sandøy was 39.4 mm for L4, 46.4 mm for L5 male and 69.6 mm for L5 female (Figure 3.2). *P. decipiens* in Hvaler had mean lengths 14 mm for L3 (one individual), 31 mm for L4, 50.2 mm for L5 male and 68.6 mm L5 female. Jensen (2009) did not differentiate between areas when discussing her results in length measurements, but found a mean of 46 mm for L5 males and 48 mm for L5 females. Aspholm (1991) found a mean of 43 mm for L5 males and 57 mm for L5 females. The t-test indicated that there were no difference between Sandøy and Hvaler for stage L4 (P=0.388). L5 male showed no difference (P=0.321), and neither did L5 female (P=0.881). *P. decipiens* stage L3 was only found in Hvaler and it was not possible to do any comperative statistical analysis.

McClelland (1980) found sexually mature *P. decipiens* females mean length to be 60.8 mm, and L5 males 54.3 mm. These numbers are closer to results in this study regarding L5 male, than Jensen (2009) and Aspholm (1991) results. McClelland (1980) found that sexually mature *P. decipiens* were significantly longer in grey seals than in harbour seals, and that the females in grey seals stomach had a much higher fecundity. Several investigations have shown that *P. decipiens* had higher abundance in grey seals than in harbour seals (Bjørge 1987b; Ólafsdóttir and Hauksson 1998; Stobo *et al.* 2002). This might indicate that *P. decipiens* would prefer grey seals over harbour seals.

McClelland (1980) investigated pathological effects of *P. decipiens* in free-living harbour seals and grey seals from Nova Scotia. These harbour seals containes on average 62 *P. decipiens*, while the grey seals had 577 individuals. Harbour seals and grey seals in captivity were infected with *P. decipiens* from cod. Six of the eight harbour seals on average had an infection on 34 *P. decipiens*, while the two last contain 250 and 547. *P. decipiens* in free-living harbour seals and grey seals have smaller pathological effect than seals in captivity. Aspholm´s (1991) theory was that seals in freedom have a diet that satisfies the nutritional demands of *P. decipiens* far better than those in captivity. Since the frequencies of host meals in captivity are out of sync with the nematode feeding periods, they start to attack the abdominal wall. McClelland (1980) does not state what kind of diet or meal times the seals in his investigations had.

The mean length of *C. osculatum* in harbour seals in Hvaler was 21.1 mm for L4, 37.6 mm for L5 male and 48 mm L5 female (Figure 3.6). There was only one finding of *C. osculatum* in Sandøy archipelago, in stage L4, it was 11 mm long. Jensen´s (2009) findings for L5 female and L5 male *C. osculatum* had a mean length of 42 and 27.1 mm, but Jensen (2009) only measured one female and few males. Aspholm (1991) found larger individuals of this species; his means were 51 mm for females and 35 mm for males. Valtronen *et al*. (1988) found the mean with male to be 38 mm and 48 mm in females in grey seals from the Baltic Sea, though the amount of nematodes in their study was low (n=24). Aspholm´s numbers are closer to this report´s finding, but there were few individuals in the dataset, three females and five males.

Berland (1963) noticed that large infections of Contracaecum sp. could displace other species of nematodes. To my current knowledge it is not known how nematode species influences each other the seal stomach. *C. osculatum* in this study was only dominant in one seal, seal number four, where it was the only nematode. In the other two seals *C. osculatum* were found and made up 30.6% and 20%. Berland (1963) observation was not valid for the results in this study, at least as far as one can implement validation on such small material. Note that the material was scarce and the results not significant statistically. Aspholm (1991) suggested that there might be elements with the seals behaviour (time between meals) and biology that may affect the nematodes growth condition. He also stated that his material could not answer any of these questions, the same accounts for this study. *Contracaecum osculatum* was not testet statistically; the species was only represented in stage L5 male and L5 female in Hvaler, stage L4 was only found once in Sandøy.

4.2.3 Sexually mature *A. simplex*

Larvae from *A. simplex* are included in the prey animals, and it is common to find larvae in the third stage in stomachs of harbour seals (Aspholm 1991; Ólafsdóttir and Hauksson 1998). The most common perception is that these nematodes are not able to grow past the third stage in harbour seal stomachs. The main host for *A. simplex* is whale (Young 1972; Anderson 2006; Berland 2006), and in the Oslofjord it is harbour porpoise (*Phocoena phocoena*) that is the anticipated main host (Ugland *et al*. 2004).

Aspholm (1991) and Jensen (2009) found sexually mature *A. simplex* in harbour seals from the Hvaler archipelago. Aspholm (1991) found seven females and 20 males in 29 investigated seals (17% of the seal stomachs contained sexually mature *A. simplex*). Jensen (2009) only investigated five harbour seals from the same area, and found sexually mature *A. simplex* in one of these. There are no sexually mature *A. simplex* in the study. Stobo *et al.* (2002) found 360 larvae, and no sexually mature *A. simplex* in 69 harbour seals near Sable Island. Brattey and Stenson (1993) found that 32% of 47 investigated harbour seals from Newfoundland and Labrador were infected with *A. simplex*, but none were sexually mature. Of 95 harbour seals investigated in Sable Island, no one contained sexually mature indivuals of *A.simplex* (Ólafsdóttir and Hauksson 1998). These comprehensive investigations in Canada and Europe imply that the findings in this study are common. In Jensen (2009) findings, all the sexually mature females had eggs in their uterus. This shows that the nematodes reproduce in harbour seals, something that had not been confirmed until recently (Jensen 2009).

Aspholm (1991) suggested that less nutrition could lead to lesser growth of the nematodes and as a result of this, staying in the sexually immature stages longer than normal. This and the hypothesis regarding “dauer larvae” - that young nematodes can be stopped in their development if there is a breach in the access for nutrition (Maupas 1899, quoted in Anderson 1983; in Aspholm 1991), could also lead to lesser growth of the nematodes. This hypothesis needs more research to be confirmed.

4.3 Heartworms

One heartworm, *Acanthocheilonema spirocauda,* of length 91 mm was found in seal number five from Sandøy archipelago. The seal was a male yearling (approximately three months old), and was in good health with blubber thickness of 29 mm (abdominal blubber) and 27 mm (chest blubber).

Harbour seals in Sweden and Denmark were investigated for *A. spirocauda*. They had a prevalence of 11.4% (18 of 158 seals) in Sweden and 8.8% (23 of 238 seals) in Denmark (Lunneryd 1992; Leidenberger and Boström 2008). Seals from Sandøy had a prevalence of 20% *A. spirocauda*. In this study only one heartworm was found, therefore the results cannot be compared to their previous investigations. Heartworm is predominantly reported in young and immature individuals (Borgsteede *et al.* 1991; Claussen *et al.* 1991; Measures *et al.* 1997; Leidenberger *et al.* 2007). Lunneryd (1992) found that 69% of heartworm in harbour seals in Skagerak was in seals younger than four years old. Investigations of *A. spirocauda* in Norway have not been completed before, as Jensens´ (2009) findings in three seals, and the material in this study are both far too small to compare with investigations done other places with more comprehensive material.

It is presumed that harbour seals are infected with *A. spirocauda* through seal lice (Anderson 2006; Leidenberger *et al*. 2007). No seal lice were observed on the seal that had heartworm. Several studies have investigated seal lice for larvae of heartworm, but only one has managed to prove the larvae stage of *A. spirocauda* in the seal lice (Geraci *et al*. 1981). Mosquito and gnat have been proposed as other intermediate hosts for the heartworm, they are known to mature heartworm in dogs and cats (Anderson 2006). They are also often observed in high density on the resting sites for harbour seals (Taylor *et al.* 1961).

4.4 Lungworms

Two lungworm species occur in seals in the North Atlantic, *Otostrongilus circumlitus* og *Parafilaroides gymnurus* (Measures 2001). Four individuals of the species *Otostrongilus circumlitus* was found in seal number four from Sandøy archipelago. The seal was a yearling (approximately three months old), and had blubber thickness of 25 mm (abdominal blubber) and 25 mm (chest blubber) indicating good condition (Drescher 1979; Bäcklin *et al*. 2010).

Infection of *O. circumlitus* occurs predominantly in seals younger than one year old (Onderka 1989; Bergeron *et al*. 1997b; Gosselin *et al.* 1998; Measures 2001). No investigations have been completed on *O. circumlitus* in coastal seal species in Norway. On the coast of California it was discovered a prevalence of 12% (37 of 304 seals), and infection rate between 1 and 280 nematodes in year old harbour seals (Gulland 1997). In Canadian harbour seals there were found a prevalence of 6% (Gosselin *et al.* 1998), and Claussen (1991) reported a prevalence of 26% in harbour seals in the Wadden Sea. These observations are built on investigations of stranded or hunted seal; therefore these young animals are overrepresented in the dataset (Gosselin *et al*. 1998; Measures 2001). According to Morten Bronndal (pers. comm 2012) younger seals are more curious and have had less exposure to humans. That may explain some of the reasons why younger seals get caught during hunting. The seals that were caught during this study with lungworms, were approximately three months old (Table 3.1). According to the information above, there seems to be a trend of lungworm in younger seals.

In an investigation of 149 harbour seals from Kattegat, Skagerak and the Baltic Sea, Lunneryd (1992) did not find *O. circumlitus* or *P. gymnurus* in the seals. In the Wadden sea, 355 harbour seals were investigated and it was found that between 37 and 57 % of the seals were infected with *O. circumlitus* and/or *P. gymnurus*, but they did not differentiate between the two species (Siebert *et al.* 2007). Clausen (1977) found that 23% of 65 harbour seals in Denmark at the end of the seventies, either died of lung infection, or they would have died of infection if they were not shot. All of these seals had lungworms either in their feces or in the lung tissue. According to Clausen (1977), all of the lungworms belonged to the species *O. circumlitus*, but Measures (2001) claims that it is more likely that larvae found in the actual lung tissue, belongs to *Parafilaroides.*

4.5 Conclusions

The purpose of investigating the nematode fauna in the seal stomachs was to be able to compare the results from this study with previous studies to find out if there were anything particular with the infection pattern of any of the stomach nematodes. There are large regional differences in mean and species composition of the stomach nematodes (Young 1972; Bjørge 1987b; Aspholm 1991; Brattey and Stenson 1993; Stobo *et al.* 2002; Hansen and Malmstrøm 2006). Aspholm (1991) also showed that infection rate varies largely from seals and within the same geographical area. There might also be indications that the population level of *A. simplex* has declined significantly since the end of the 1980s´(Jensen 1987; Hansen and Malmstrøm 2006; Jensen (2009); Own results).

No firm conclusions could be made regarding differences in the prevalence, abundance and intensity in *Pseudoterranova decipiens*, *Anisakis simplex* and *Contracaecum osculatum* in Sandøy and Hvaler archipelago, due to small sample sizes. One could neither draw any conclusions regarding differences in sex, stage and length in the same three nematode species from Sandøy and Hvaler archipelago. However, there might be a pattern in this study showing that there are more *P. decipiens* in seals from Hvaler and Sandøy than the other two common nematode species *A. simplex* and *C. osculatum*.

There were no sexually mature *A. simplex* in the stomachs of the harbour seals from this study. It is not common that *A. simplex* grows to the fourth and fifth stage in the stomachs of harbour seals (Young 1972; Brattey and Stenson 1993; Ólafsdóttir and Hauksson 1998; Stobo *et al.* 2002). The results from this study are thus regarded as the norm, with few *A. simplex* in stage L4 and no findings of *A. simplex* in stage L5.

There were harbour seals from Sandøy archipelago with heart- and lungworm. None of the seals from Hvaler archipelago in this study was infected with heart- and lungworm. According to my knowledge, there have not been any investigations of lungworm (*O. circumlitus* and *P. gymnurus)* and heartworm (*A. spirocauda*) in Norway earlier. Unfortunately, the material in this study is not large enough to draw any conclusions about these nematodes in harbour seals in Norway. There might be an indication of younger seals having a heavier burden of heart- and lungworms. The seals from this study were yearlings, and no older seals showed signs of heart- and lungworms. Again the small sample size and infection rate in this study might have influenced this result.

Infection of stomach nematodes does not seem to be of great influence on the seal's health (Geraci and Aubin 1987). It has been proposed that these nematodes and the infected seal can benefit from each other; the nematode helps break down the nutrition in the stomachs of the seals (Berland 2006). It may seem that seals can have moderate infections of heart- and lungworm without influencing the condition of the seal (Gosselin *et al*. 1998; Jensen 2009). However, powerful infections of heart- and lung nematode have caused deadly pneumonia (Van den Broek and Wensvoort 1959; Fleischmann and Squire 1970; Clausen 1977; Stroud and Dailey 1978; Breuer *et al.* 1988a; Gulland *et al.* 1997; Vercruysse *et al.* 2003).

4.6 Future directions

For future perspectives I would recommend to increase the seal sample size in order to be able to analyze the datamaterial statistically and see trends regarding nematode species distribution. Like Karl Inne Ugland (pers. comm. 2012), I would also recommend to aim for an optimal sample size of 50 or more seals to be able to get a normal distribution of the stomach nematodes. It would be preferable to have a material of both young and adult individuals to investigate if the nematodes are distributed in a certain respect due to age. Data material consisting of both young and adult seals might also show a certain pattern regarding heart- and lungworm prevalence, abundance and intensity. The Phocine Distemper Virus (PDV) heavily reduced the harbour seal population around Hvaler archipelago in 1988 and 2002 (Markussen 1992; Morten Bronndal, pers. comm. In Jensen 2009). If one can identify the heart- and lungworm distribution, perhaps one can learn the reasons why so many harbour seals died during the PDV plague in 1988 and 2002.

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Appendix A: Analytical data

Nematode #	Seal #	Area #	Dato	Seal sex	Nematode sex	Stage	Lenght (mm)	Species	Place collected
1	1	Sandøy	02.06.2010	female		L4	13	Pseudoterranova decipiens	Stomach
2	1	Sandøy	02.06.2010	female		L4	43	Pseudoterranova decipiens	Stomach
3	1	Sandøy	02.06.2010	female	Male	L5	48	Pseudoterranova decipiens	Stomach
4	1	Sandøy	02.06.2010	female	Male	L5	59	Pseudoterranova decipiens	Stomach
5	1	Sandøy	02.06.2010	female	Male	L5	50	Pseudoterranova decipiens	Stomach
6	1	Sandøy	02.06.2010	female	Male	L5	62	Pseudoterranova decipiens	Stomach
7	1	Sandøy	02.06.2010	female	Male	L5	49	Pseudoterranova decipiens	Stomach
8	1	Sandøy	02.06.2010	female	Male	L5	32	Pseudoterranova decipiens	Stomach
9	1	Sandøy	02.06.2010	female	Male	L5	41	Pseudoterranova decipiens	Stomach
10	1	Sandøy	02.06.2010	female	Male	L5	45	Pseudoterranova decipiens	Stomach
11	1	Sandøy	02.06.2010	female	Male	L5	50	Pseudoterranova decipiens	Stomach
12	1	Sandøy	02.06.2010	female	Male	L5	55	Pseudoterranova decipiens	Stomach
13	1	Sandøy	02.06.2010	female	Male	L5	50	Pseudoterranova decipiens	Stomach
14	1	Sandøy	02.06.2010	female	Male	L5	46	Pseudoterranova decipiens	Stomach
15	1	Sandøy	02.06.2010	female	Female	L5	69	Pseudoterranova decipiens	Stomach
16	1	Sandøy	02.06.2010	female	Female	L5	72	Pseudoterranova decipiens	Stomach
17	1	Sandøy	02.06.2010	female	Female	L5	60	Pseudoterranova decipiens	Stomach
18	1	Sandøy	02.06.2010	female	Female	L5	63	Pseudoterranova decipiens	Stomach
19	1	Sandøy	02.06.2010	female	Female	L5	60	Pseudoterranova decipiens	Stomach
20	1	Sandøy	02.06.2010	female	Female	L5	69	Pseudoterranova decipiens	Stomach
21	1	Sandøy	02.06.2010	female	Female	L5	60	Pseudoterranova decipiens	Stomach
22	2	Sandøy	03.06.2010	Male		L4	22	Anisakis simplex	Stomach
23	2	Sandøy	03.06.2010	Male		L4	18	Anisakis simplex	Stomach
24	2	Sandøy	03.06.2010	Male		L4	19	Anisakis simplex	Stomach

25	2	Sandøy	03.06.2010	Male		L4	23	Anisakis simplex	Stomach
26	2	Sandøy	03.06.2010	Male		L4	29	Anisakis simplex	Stomach
27	2	Sandøy	03.06.2010	Male		L4	24	Anisakis simplex	Stomach
28	2	Sandøy	03.06.2010	Male		L4	34	Anisakis simplex	Stomach
29	2	Sandøy	03.06.2010	Male		L4	50	Pseudoterranova decipiens	Chest cavity
30	2	Sandøy	03.06.2010	Male		L4	51	Pseudoterranova decipiens	Chest cavity
31	2	Sandøy	03.06.2010	Male		L4	32	Pseudoterranova decipiens	Chest cavity
32	2	Sandøy	03.06.2010	Male	Male	L5	51	Pseudoterranova decipiens	Stomach
33	2	Sandøy	03.06.2010	Male	Male	L5	50	Pseudoterranova decipiens	Stomach
34	2	Sandøy	03.06.2010	Male	Male	L5	36	Pseudoterranova decipiens	Stomach
35	2	Sandøy	03.06.2010	Male	Male	L5	36	Pseudoterranova decipiens	Stomach
36	2	Sandøy	03.06.2010	Male	Male	L5	52	Pseudoterranova decipiens	Stomach
37	2	Sandøy	03.06.2010	Male	Male	L5	33	Pseudoterranova decipiens	Stomach
38	2	Sandøy	03.06.2010	Male	Male	L5	40	Pseudoterranova decipiens	Stomach
39	2	Sandøy	03.06.2010	Male	Male	L5	47	Pseudoterranova decipiens	Stomach
40	2	Sandøy	03.06.2010	Male	Male	L5	45	Pseudoterranova decipiens	Stomach
41	2	Sandøy	03.06.2010	Male	Female	L5	82	Pseudoterranova decipiens	Stomach
42	2	Sandøy	03.06.2010	Male	Female	L5	64	Pseudoterranova decipiens	Stomach
43	2	Sandøy	03.06.2010	Male	Female	L5	72	Pseudoterranova decipiens	Stomach
44	2	Sandøy	03.06.2010	Male	Female	L5	76	Pseudoterranova decipiens	Stomach
45	2	Sandøy	03.06.2010	Male	Female	L5	75	Pseudoterranova decipiens	Stomach
46	2	Sandøy	03.06.2010	Male	Female	L5	71	Pseudoterranova decipiens	Stomach
47	2	Sandøy	03.06.2010	Male	Female	L5	68	Pseudoterranova decipiens	Stomach
48	2	Sandøy	03.06.2010	Male	Female	L5	81	Pseudoterranova decipiens	Stomach
49	2	Sandøy	03.06.2010	Male	Female	L5	61	Pseudoterranova decipiens	Stomach
50	2	Sandøy	03.06.2010	Male	Female	L5	70	Pseudoterranova decipiens	Stomach
51	2	Sandøy	03.06.2010	Male	Female	L5	52	Pseudoterranova decipiens	Stomach

52	2	Sandøy	03.06.2010	Male	Female	L5	69	Pseudoterranova decipiens	Stomach
53	3	Sandøy	05.06.2010	Female		L3	30	Anisakis simplex	Stomach
54	3	Sandøy	05.06.2010	Female		L3	27	Anisakis simplex	Stomach
55	3	Sandøy	05.06.2010	Female		L3	20	Anisakis simplex	Stomach
56	3	Sandøy	05.06.2010	Female		L4	41	Anisakis simplex	Stomach
57	3	Sandøy	05.06.2010	Female		L4	46	Pseudoterranova decipiens	Stomach
58	3	Sandøy	05.06.2010	Female		L4	50	Pseudoterranova decipiens	Stomach
59	3	Sandøy	05.06.2010	Female		L4	51	Pseudoterranova decipiens	Stomach
60	3	Sandøy	05.06.2010	Female	Male	L5	45	Pseudoterranova decipiens	Stomach
61	3	Sandøy	05.06.2010	Female	Male	L5	59	Pseudoterranova decipiens	Stomach
62	3	Sandøy	05.06.2010	Female	Male	L5	51	Pseudoterranova decipiens	Stomach
63	3	Sandøy	05.06.2010	Female	Male	L5	50	Pseudoterranova decipiens	Stomach
64	3	Sandøy	05.06.2010	Female	Male	L5	52	Pseudoterranova decipiens	Stomach
65	3	Sandøy	05.06.2010	Female	Female	L5	80	Pseudoterranova decipiens	Stomach
66	3	Sandøy	05.06.2010	Female	Female	L5	88	Pseudoterranova decipiens	Stomach
67	3	Sandøy	05.06.2010	Female	Female	L5	70	Pseudoterranova decipiens	Stomach
68	3	Sandøy	05.06.2010	Female	Female	L5	65	Pseudoterranova decipiens	Stomach
69	3	Sandøy	05.06.2010	Female	Female	L5	64	Pseudoterranova decipiens	Stomach
70	3	Sandøy	05.06.2010	Female	Female	L5	79	Pseudoterranova decipiens	Stomach
71	4	Hvaler	18.08.2010	Male		L3	22	Anisakis simplex	Stomach
72	4	Hvaler	18.08.2010	Male		L3	25	Anisakis simplex	Stomach
73	4	Hvaler	18.08.2010	Male		L3	11	Anisakis simplex	Stomach
74	4	Hvaler	18.08.2010	Male		L3	21	Anisakis simplex	Stomach
75	4	Hvaler	18.08.2010	Male		L3	23	Anisakis simplex	Stomach
76	4	Hvaler	18.08.2010	Male		L3	26	Anisakis simplex	Stomach
77	4	Hvaler	18.08.2010	Male		L3	27	Anisakis simplex	Stomach
78	4	Hvaler	18.08.2010	Male		L3	20	Anisakis simplex	Stomach
79	4	Hvaler	18.08.2010	Male		L3	22	Anisakis simplex	Stomach
80	4	Hvaler	18.08.2010	Male		L3	23	Anisakis simplex	Stomach
81	4	Hvaler	18.08.2010	Male		L3	24	Anisakis simplex	Stomach
82	4	Hvaler	18.08.2010	Male		L3	17	Anisakis simplex	Stomach
83	4	Hvaler	18.08.2010	Male		L3	21	Anisakis simplex	Stomach
84	4	Hvaler	18.08.2010	Male		L3	20	Anisakis simplex	Stomach
85	4	Hvaler	18.08.2010	Male		L3	19	Anisakis simplex	Stomach
86	4	Hvaler	18.08.2010	Male		L3	16	Anisakis simplex	Stomach

87	4	Hvaler	18.08.2010	Male		L3	17	Anisakis simplex	Stomach
88	4	Hvaler	18.08.2010	Male		L3	21	Anisakis simplex	Stomach
89	4	Hvaler	18.08.2010	Male		L4	33	Contracaecum osculatum	Stomach
90	4	Hvaler	18.08.2010	Male		L4	23	Contracaecum osculatum	Stomach
91	4	Hvaler	18.08.2010	Male		L4	36	Contracaecum osculatum	Stomach
92	4	Hvaler	18.08.2010	Male		L4	34	Contracaecum osculatum	Stomach
93	4	Hvaler	18.08.2010	Male		L4	13	Contracaecum osculatum	Stomach
94	4	Hvaler	18.08.2010	Male		L4	13	Contracaecum osculatum	Stomach
95	4	Hvaler	18.08.2010	Male	Female	L5	61	Contracaecum osculatum	Stomach
96	4	Hvaler	18.08.2010	Male	Female	L5	43	Contracaecum osculatum	Stomach
97	4	Hvaler	18.08.2010	Male	Female	L5	40	Contracaecum osculatum	Stomach
98	4	Hvaler	18.08.2010	Male	Male	L5	49	Contracaecum osculatum	Stomach
99	4	Hvaler	18.08.2010	Male	Male	L5	39	Contracaecum osculatum	Stomach
100	4	Hvaler	18.08.2010	Male		L4	56	Pseudoterranova decipiens	Stomach
101	4	Hvaler	18.08.2010	Male		L4	23	Pseudoterranova decipiens	Stomach
102	4	Hvaler	18.08.2010	Male		L4	32	Pseudoterranova decipiens	Stomach
103	4	Hvaler	18.08.2010	Male		L4	33	Pseudoterranova decipiens	Stomach
104	4	Hvaler	18.08.2010	Male	Female	L5	79	Pseudoterranova decipiens	Stomach
105	4	Hvaler	18.08.2010	Male	Male	L5	47	Pseudoterranova decipiens	Stomach
106	4	Hvaler	18.08.2010	Male	Male	L5	41	Pseudoterranova decipiens	Stomach
107	5	Hvaler	22.09.2010	Male		L4	28	Contracaecum osculatum	Stomach
108	5	Hvaler	22.09.2010	Male		L4	10	Contracaecum osculatum	Stomach
109	5	Hvaler	22.09.2010	Male		L4	13	Contracaecum osculatum	Stomach
110	5	Hvaler	22.09.2010	Male		L4	12	Contracaecum osculatum	Stomach
111	5	Hvaler	22.09.2010	Male		L4	17	Contracaecum osculatum	Stomach
112	5	Hvaler	22.09.2010	Male	Male	L5	32	Contracaecum osculatum	Stomach

113	5	Hvaler	22.09.2010	Male	Male	L5	37	Contracaecum osculatum	Stomach
114	5	Hvaler	22.09.2010	Male	Male	L5	31	Contracaecum osculatum	Stomach
115	5	Hvaler	22.09.2010	Male		L3	14	Anisakis simplex	Stomach
116	5	Hvaler	22.09.2010	Male		L3	11	Anisakis simplex	Stomach
117	5	Hvaler	22.09.2010	Male		L3	11	Anisakis simplex	Stomach
118	5	Hvaler	22.09.2010	Male		L3	12	Anisakis simplex	Stomach
119	5	Hvaler	22.09.2010	Male		L3	17	Anisakis simplex	Stomach
120	5	Hvaler	22.09.2010	Male		L3	17	Anisakis simplex	Stomach
121	5	Hvaler	22.09.2010	Male		L3	13	Anisakis simplex	Stomach
122	5	Hvaler	22.09.2010	Male		L3	15	Anisakis simplex	Stomach
123	5	Hvaler	22.09.2010	Male		L3	18	Anisakis simplex	Stomach
124	5	Hvaler	22.09.2010	Male		L3	20	Anisakis simplex	Stomach
125	5	Hvaler	22.09.2010	Male		L3	14	Anisakis simplex	Stomach
126	5	Hvaler	22.09.2010	Male		L4	12	Anisakis simplex	Stomach
127	5	Hvaler	22.09.2010	Male		L4	17	Anisakis simplex	Stomach
128	5	Hvaler	22.09.2010	Male		L4	11	Anisakis simplex	Stomach
129	5	Hvaler	22.09.2010	Male		L3	18	Anisakis simplex	Rectum
130	5	Hvaler	22.09.2010	Male		L3	15	Anisakis simplex	Rectum
131	5	Hvaler	22.09.2010	Male		L3	16	Anisakis simplex	Rectum
132	5	Hvaler	22.09.2010	Male		L3	14	Anisakis simplex	Rectum
133	5	Hvaler	22.09.2010	Male		L3	12	Anisakis simplex	Rectum
134	5	Hvaler	22.09.2010	Male		L3	16	Anisakis simplex	Rectum
135	5	Hvaler	22.09.2010	Male		L3	13	Anisakis simplex	Rectum
136	5	Hvaler	22.09.2010	Male		L3	12	Anisakis simplex	Rectum
137	5	Hvaler	22.09.2010	Male		L3	14	Anisakis simplex	Rectum
138	5	Hvaler	22.09.2010	Male		L4	29	Pseudoterranova decipiens	Stomach
139	5	Hvaler	22.09.2010	Male		L4	30	Pseudoterranova decipiens	Stomach
140	5	Hvaler	22.09.2010	Male		L3	14	Pseudoterranova decipiens	Stomach
141	5	Hvaler	22.09.2010	Male	Male	L5	43	Pseudoterranova decipiens	Stomach
142	5	Hvaler	22.09.2010	Male	Male	L5	60	Pseudoterranova decipiens	Stomach
143	5	Hvaler	22.09.2010	Male	Male	L5	59	Pseudoterranova decipiens	Stomach
144	5	Hvaler	22.09.2010	Male	Male	L5	51	Pseudoterranova decipiens	Stomach
145	5	Hvaler	22.09.2010	Male	Female	L5	66	Pseudoterranova decipiens	Stomach
146	5	Hvaler	22.09.2010	Male	Female	L5	61	Pseudoterranova decipiens	Stomach
147	6	Sandøy	28.09.2010	Male		L4	11	Contracaecum osculatum	Stomach
148	6	Sandøy	28.09.2010	Male				Otostrongylus circumlitus	Lung
149	6	Sandøy	28.09.2010	Male				Otostrongylus	Lung

								circumlitus	
150	6	Sandøy	28.09.2010	Male				Otostrongylus circumlitus	Lung
151	6	Sandøy	28.09.2010	Male				Otostrongylus circumlitus	Lung
152	7	Sandøy	29.09.2010	Male			91	Acanthocheilonema spirocauda	Heart
153	7	Sandøy	29.09.2010	Male		L3	11	Anisakis simplex	Stomach
154	7	Sandøy	29.09.2010	Male		L3	15	Anisakis simplex	Stomach
155	7	Sandøy	29.09.2010	Male		L4	28	Pseudoterranova decipiens	Stomach
156	7	Sandøy	29.09.2010	Male		L4	30	Pseudoterranova decipiens	Stomach

Appendix B: Observations

Observations			
Date	Area	Number of harbour seals	Number of grey seals
03.06.2010	Ona fyr, Sandøy	Approximately 50	
22.09.2010	Hvaler	Approximately 12-15	
28.09.2010	Ona fyr, Sandøy	36	
29.09.2010	Ona fyr, Sandøy	35	
30.09.2010	Galleskjæra, Sandøy		2
27.09.2011	Torbjørnskjær, Hvaler	50	
25.10.2011	Torbjørnskjær, Hvaler	10	

Appendix C: Figures

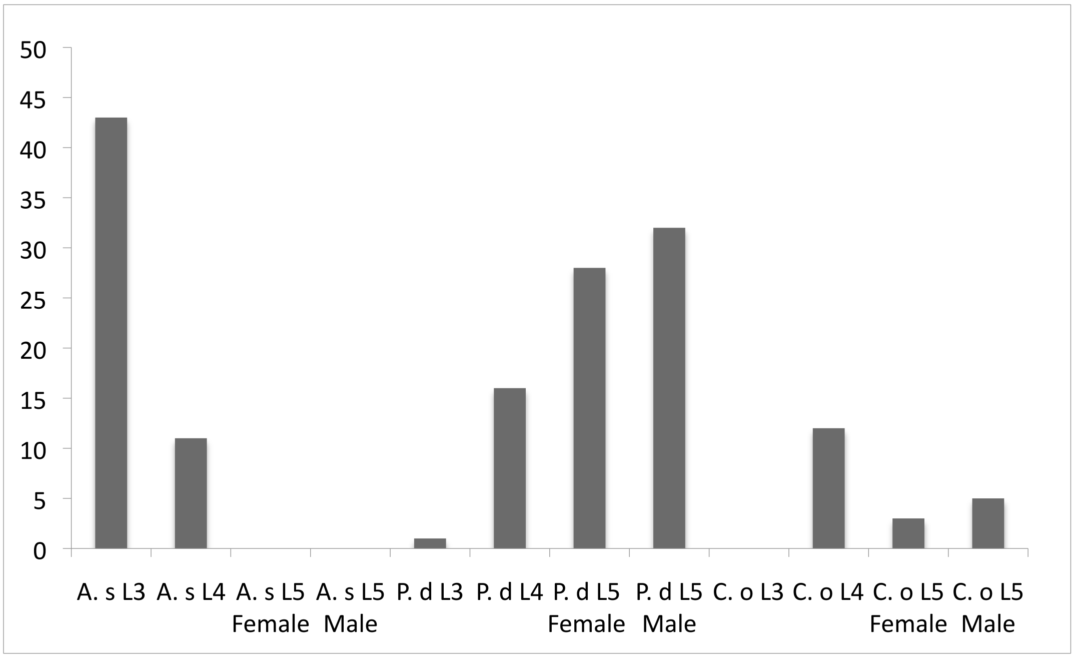

Figure X.X distribution of the stages and species of the nematodes in the seals stomach (and chest cavity + rectum). *Anisakis simplex* (A.s), *Pseudoterranova decipiens* (P.d) and *Contracaecum osculatum* (C.o).

Appendix D: Length measurement data

Area #	Stage	Lenght (mm)	Species	
Sandøy	L4	13	Pseudoterranova decipiens	1
Sandøy	L4	43	Pseudoterranova decipiens	2
Sandøy	L4	50	Pseudoterranova decipiens	3
Sandøy	L4	51	Pseudoterranova decipiens	4
Sandøy	L4	32	Pseudoterranova decipiens	5
Sandøy	L4	46	Pseudoterranova decipiens	6
Sandøy	L4	50	Pseudoterranova decipiens	7
Sandøy	L4	51	Pseudoterranova decipiens	8
Sandøy	L4	28	Pseudoterranova decipiens	9
Sandøy	L4	30	Pseudoterranova decipiens	10
		Mean 39,4	Standard deviation12,99743564	Amount=10
Sandøy	L5 Male	48	Pseudoterranova decipiens	1
Sandøy	L5 Male	59	Pseudoterranova decipiens	2
Sandøy	L5 Male	50	Pseudoterranova decipiens	3
Sandøy	L5 Male	62	Pseudoterranova decipiens	4
Sandøy	L5 Male	49	Pseudoterranova decipiens	5
Sandøy	L5 Male	32	Pseudoterranova decipiens	6
Sandøy	L5 Male	41	Pseudoterranova decipiens	7
Sandøy	L5 Male	45	Pseudoterranova decipiens	8
Sandøy	L5 Male	50	Pseudoterranova decipiens	9
Sandøy	L5 Male	55	Pseudoterranova decipiens	10
Sandøy	L5 Male	50	Pseudoterranova decipiens	11
Sandøy	L5 Male	46	Pseudoterranova decipiens	12
Sandøy	L5 Male	51	Pseudoterranova decipiens	13
Sandøy	L5 Male	50	Pseudoterranova decipiens	14
Sandøy	L5 Male	36	Pseudoterranova decipiens	15
Sandøy	L5 Male	36	Pseudoterranova decipiens	16
Sandøy	L5 Male	52	Pseudoterranova decipiens	17
Sandøy	L5 Male	33	Pseudoterranova decipiens	18
Sandøy	L5 Male	40	Pseudoterranova decipiens	19
Sandøy	L5 Male	47	Pseudoterranova decipiens	20
Sandøy	L5 Male	45	Pseudoterranova decipiens	21
Sandøy	L5 Male	51	Pseudoterranova decipiens	22
Sandøy	L5 Male	50	Pseudoterranova decipiens	23
Sandøy	L5 Male	36	Pseudoterranova decipiens	24
Sandøy	L5 Male	36	Pseudoterranova decipiens	25
Sandøy	L5 Male	52	Pseudoterranova decipiens	26
Sandøy	L5 Male	33	Pseudoterranova decipiens	27
Sandøy	L5 Male	40	Pseudoterranova decipiens	28
Sandøy	L5 Male	47	Pseudoterranova decipiens	29
Sandøy	L5 Male	45	Pseudoterranova decipiens	30
Sandøy	L5 Male	45	Pseudoterranova decipiens	31
Sandøy	L5 Male	59	Pseudoterranova decipiens	32
Sandøy	L5 Male	51	Pseudoterranova decipiens	33

Sandøy	L5 Male	50	Pseudoterranova decipiens	34
Sandøy	L5 Male	52	Pseudoterranova decipiens	35
		Mean 46,4	Standard deviation 7,666581415	Amount=35
Sandøy	L5 Female	69	Pseudoterranova decipiens	1
Sandøy	L5 Female	72	Pseudoterranova decipiens	2
Sandøy	L5 Female	60	Pseudoterranova decipiens	3
Sandøy	L5 Female	63	Pseudoterranova decipiens	4
Sandøy	L5 Female	60	Pseudoterranova decipiens	5
Sandøy	L5 Female	69	Pseudoterranova decipiens	6
Sandøy	L5 Female	60	Pseudoterranova decipiens	7
Sandøy	L5 Female	82	Pseudoterranova decipiens	8
Sandøy	L5 Female	64	Pseudoterranova decipiens	9
Sandøy	L5 Female	72	Pseudoterranova decipiens	10
Sandøy	L5 Female	76	Pseudoterranova decipiens	11
Sandøy	L5 Female	75	Pseudoterranova decipiens	12
Sandøy	L5 Female	71	Pseudoterranova decipiens	13
Sandøy	L5 Female	68	Pseudoterranova decipiens	14
Sandøy	L5 Female	81	Pseudoterranova decipiens	15
Sandøy	L5 Female	61	Pseudoterranova decipiens	16
Sandøy	L5 Female	70	Pseudoterranova decipiens	17
Sandøy	L5 Female	52	Pseudoterranova decipiens	18
Sandøy	L5 Female	69	Pseudoterranova decipiens	19
Sandøy	L5 Female	80	Pseudoterranova decipiens	20
Sandøy	L5 Female	88	Pseudoterranova decipiens	21
Sandøy	L5 Female	70	Pseudoterranova decipiens	22
Sandøy	L5 Female	65	Pseudoterranova decipiens	23
Sandøy	L5 Female	64	Pseudoterranova decipiens	24
Sandøy	L5 Female	79	Pseudoterranova decipiens	25
		Mean 69,6	Standard deviation 8,401388774	Amount=25
Sandøy	L3	30	Anisakis simplex	1
Sandøy	L3	27	Anisakis simplex	2
Sandøy	L3	20	Anisakis simplex	3
Sandøy	L3	11	Anisakis simplex	4
Sandøy	L3	15	Anisakis simplex	5
		Mean 20,6	Standard deviation 7,956129712	Amount=5
Sandøy	L4	22	Anisakis simplex	1
Sandøy	L4	18	Anisakis simplex	2
Sandøy	L4	19	Anisakis simplex	3
Sandøy	L4	23	Anisakis simplex	4
Sandøy	L4	29	Anisakis simplex	5
Sandøy	L4	24	Anisakis simplex	6
Sandøy	L4	34	Anisakis simplex	7
Sandøy	L4	41	Anisakis simplex	8
		Mean 26,25	Standard deviation 7,923743704	Amount=8
Sandøy	L4	11	Contracaecum osculatum	

Hvaler	L3	14	Pseudoterranova decipiens	1
Hvaler	L4	56	Pseudoterranova decipiens	1
Hvaler	L4	23	Pseudoterranova decipiens	2
Hvaler	L4	32	Pseudoterranova decipiens	3
Hvaler	L4	33	Pseudoterranova decipiens	4
Hvaler	L4	29	Pseudoterranova decipiens	5
Hvaler	L4	30	Pseudoterranova decipiens	6
		Mean 31	Standard deviation 12,83225104	Amount =6
Hvaler	L5 Male	43	Pseudoterranova decipiens	1
Hvaler	L5 Male	60	Pseudoterranova decipiens	2
Hvaler	L5 Male	59	Pseudoterranova decipiens	3
Hvaler	L5 Male	51	Pseudoterranova decipiens	4
Hvaler	L5 Male	47	Pseudoterranova decipiens	5
Hvaler	L5 Male	41	Pseudoterranova decipiens	6
		Mean 50,16666667	Standard deviation 8,010409894	Amount=6
Hvaler	L5 Female	79	Pseudoterranova decipiens	1
Hvaler	L5 Female	66	Pseudoterranova decipiens	2
Hvaler	L5 Female	61	Pseudoterranova decipiens	3
		Mean 68,66666667	Standard deviation 9,291573243	Amount=3
Hvaler	L3	22	Anisakis simplex	1
Hvaler	L3	25	Anisakis simplex	2
Hvaler	L3	11	Anisakis simplex	3
Hvaler	L3	21	Anisakis simplex	4
Hvaler	L3	23	Anisakis simplex	5
Hvaler	L3	26	Anisakis simplex	6
Hvaler	L3	27	Anisakis simplex	7
Hvaler	L3	20	Anisakis simplex	8
Hvaler	L3	22	Anisakis simplex	9
Hvaler	L3	23	Anisakis simplex	10
Hvaler	L3	24	Anisakis simplex	11
Hvaler	L3	17	Anisakis simplex	12
Hvaler	L3	21	Anisakis simplex	13
Hvaler	L3	20	Anisakis simplex	14
Hvaler	L3	19	Anisakis simplex	15
Hvaler	L3	16	Anisakis simplex	16
Hvaler	L3	17	Anisakis simplex	17
Hvaler	L3	21	Anisakis simplex	18
Hvaler	L3	14	Anisakis simplex	19
Hvaler	L3	11	Anisakis simplex	20
Hvaler	L3	11	Anisakis simplex	21
Hvaler	L3	12	Anisakis simplex	22
Hvaler	L3	17	Anisakis simplex	23
Hvaler	L3	17	Anisakis simplex	24

Hvaler	L3	13	Anisakis simplex	25
Hvaler	L3	15	Anisakis simplex	26
Hvaler	L3	18	Anisakis simplex	27
Hvaler	L3	20	Anisakis simplex	28
Hvaler	L3	14	Anisakis simplex	29
Hvaler	L3	18	Anisakis simplex	30
Hvaler	L3	15	Anisakis simplex	31
Hvaler	L3	16	Anisakis simplex	32
Hvaler	L3	14	Anisakis simplex	33
Hvaler	L3	12	Anisakis simplex	34
Hvaler	L3	16	Anisakis simplex	35
Hvaler	L3	13	Anisakis simplex	36
Hvaler	L3	12	Anisakis simplex	37
Hvaler	L3	14	Anisakis simplex	38
		Mean 17,55263158	Standard deviation 4,494425186	Amount=38
Hvaler	L4	12	Anisakis simplex	1
Hvaler	L4	17	Anisakis simplex	2
Hvaler	L4	11	Anisakis simplex	3
		Mean 13,33333333	Standard deviation 3,214550254	Amount=3
Hvaler	L4	33	Contracaecum osculatum	1
Hvaler	L4	23	Contracaecum osculatum	2
Hvaler	L4	36	Contracaecum osculatum	3
Hvaler	L4	34	Contracaecum osculatum	4
Hvaler	L4	13	Contracaecum osculatum	5
Hvaler	L4	13	Contracaecum osculatum	6
Hvaler	L4	28	Contracaecum osculatum	7
Hvaler	L4	10	Contracaecum osculatum	8
Hvaler	L4	13	Contracaecum osculatum	9
Hvaler	L4	12	Contracaecum osculatum	10
Hvaler	L4	17	Contracaecum osculatum	11
		Mean 21,09090909	Standard deviation 10,00454442	Amount=11
Hvaler	L5 Male	32	Contracaecum osculatum	1
Hvaler	L5 Male	37	Contracaecum osculatum	2
Hvaler	L5 Male	31	Contracaecum osculatum	3
Hvaler	L5 Male	49	Contracaecum osculatum	4
Hvaler	L5 Male	39	Contracaecum osculatum	5
		Mean 37,6	Standard deviation 7,197221686	Amount=5
Hvaler	L5 Female	61	Contracaecum osculatum	1
Hvaler	L5 Female	43	Contracaecum osculatum	2
Hvaler	L5 Female	40	Contracaecum osculatum	3
		Mean 48	Standard deviation 11,35781669	Amount=3

Appendix E: Lungworm data

ID	From seal #	Area#	Stage	Sex	Species	Origin	Notes
1	6	Sandøy	Adult	Male	Otostrongylus circumlitus	Lung	Not complete, but found enough pieces
2	6	Sandøy	Adult	Female	Otostrongylus circumlitus	Lung	Coiled up when it died, split in two because of the coil that coiled when it died. Found enough pieces.
3	6	Sandøy	Adult		Otostrongylus circumlitus	Lung	Not complete, but found enough pieces
4	6	Sandøy	Adult		Otostrongylus circumlitus	Lung	Not complete, but found enough pieces